[Frontiers in Bioscience 9, 1339-1347, May 1, 2004]

INFLAMMATORY RESPONSES TO ISCHEMIA AND REPERFUSION IN THE CEREBRAL MICROCIRCULATION

Mami Ishikawa, John H. Zhang, Anil Nanda and D. Neil Granger

*Departments of Molecular and Cellular Physiology, and *Neurosurgery, Louisiana State University Health Sciences Center, Shreveport*

TABLE OF CONTENTS

1. ABSTRACT

Ischemia and reperfusion (I/R) has been shown to elicit an inflammatory response that is characterized by an increased production of reactive oxygen species, and the rolling, firm adhesion, and transendothelial migration of leukocytes in postcapillary venules. A rate-determining role for leukocyte-endothelial cell (L/E) adhesion in the initiation and propagation of reperfusion injury is supported by several reports that describe attenuated microvascular dysfunction and tissue injury following I/R in animals receiving neutralizing antibodies directed against certain leukocyte adhesion receptors and in mutant mice that are genetically deficient in these adhesion receptors The technique of intravital videomicroscopy has been applied to several tissues, including the brain, in order to directly observe the microcirculatory alterations and inflammatory responses that are elicited by I/R. The leukocyte- and platelet-endothelial cell adhesion responses to cerebral I/R are addressed and compared to responses observed in other postischemic vascular beds. The limited data available for the brain microcirculation support the potential of anti-leukocyte and anti-platelet strategies for stroke therapy.

2. INTRODUCTION

Ischemic stroke, a disease with high mortality worldwide, is not easily prevented prior to the appearance of the symptoms. As reported for other tissues, the severity of neuronal injury caused by ischemia is related to both the magnitude and duration of the ischemic insult (1). When blood flow is restored to the ischemic brain, reperfusion injury can occur except in the infarcted area, where neuronal function usually cannot recover due to microvascular dysfunction. Preclinical studies have revealed that the sudden restoration of blood flow to an ischemic brain can initiate a cascade of events that ultimately results in an acute and potentially injurious inflammatory reaction. In the microcirculation, this inflammatory response is manifested as the rolling, firm adhesion, and transendothelial migration of leukocytes in postcapillary venules, endothelial barrier dysfunction in both capillaries and venules, and an increased production of reactive oxygen species within all segments (arterioles, capillaries & venules) of the microvasculature. Leukocyte accumulation in whole brain tissue after ischemia is also consistent with an inflammatory response (2). Despite the deleterious effects of reperfusion, blood flow restoration to the ischemic brain is essential for survival of the patient.

Villringer et al. first described the adhesion of leukocytes in the postischemic microvasculature of the brain. Leukocyte-endothelial cell interactions within the brain microcirculation have also been examined after exposure to other stimuli, including LTB_4 stimulation (3,4), bacterial meningitis (5), traumatic brain injury (6), PAF-stimulation (7,8) and TNF-alpha stimulation (9). In this review, emphasis is given to evaluating the available information concerning the microvascular and inflammatory responses of the cerebral microcirculation to

ischemia and reperfusion. The major objectives of this review are 1) to describe the intravital microscopic methods used to observe the cerebral microcirculation; 2) to summarize the behavior of leukocytes and platelets in cerebral venules exposed to ischemia and reperfusion (I/R), and 3) to address the mechanisms that underlie the inflammatory and prothrombogenic responses of the brain to I/R.

3. TECHNICAL CONSIDERATIONS

3.1. Cranial window

Since Morii et al. (10) provided the first description of a closed cranial window for studies of the cerebral microcirculation in small animals (rat, mouse, gerbil and newborn pig), intravital microscopy has been routinely applied to studies of the brain. While the cranial window technique is a relatively atraumatic method, there is a necessity to cut the dura mater for observation of brain microvessels in the rat, but not in the gerbil (11) or mouse (12), except when superfusion of a reagent over brain surface is required. The dura mater of rat brain is richly vascularized, making it difficult to visualize microvessels in the underlying brain tissue. The highly vascularized dura mater of the rat necessitates the use of a fine bipolar coagulator to cut the tissue without excessive bleeding. This can be achieved with the use of a stereo microscope and bright light illumination. The low vascular density in mouse dura mater, eliminates the need to cut the tissue for observation of the cerebral microcirculation in mice (12) and gerbils (11).

An addition to cutting the dura, removal of the bone flap should be performed very carefully in order to create a cranial window without brain damage. A drill is used to create a donut shaped flap that is removed without inserting forceps beneath the bone during its removal. If properly performed, the bone flap and dura mater are prepared without eliciting the adhesion of leukocytes in the underlying cerebral venules.

3.2. Intravital fluorescence microscopy

Unlike for transparent tissues such as the mesentery or mouse cremaster muscle, the brain microcirculation cannot be visualized using conventional light microscopy because the density of this tissue does not allow for light to pass through it. Hence, visualization of events that occur within cerebral microvessels necessitates the use of fluorochromes and intravital fluorescence microscopy.

Leukocytes are usually observed in the cerebral microcirculation by administration of the fluorochrome rhodamine 6G (absorption peak, 525 nm; emission peak 555 nm), which are taken up by circulating leukocytes. Continuous infusion (rather than single bolus injection) of rhodamine 6G produce clear and sharp images of leukocytes. Flow cytometry has revealed that essentially 100% of leukocytes are stained by intravenous rhodamine 6G (13). We have used carboxyfluorescein diacetate succinimidyl ester (CFDASE) (12) for ex vivo labeling of platelets. CFDASE forms the stable fluorochrome carboxyfluorescein succinimidyl ester (CFSE, absorption peak 492 nm, emission peak 518 nm) after reaction with intracellular esterases.

As a light source, xenon or mercury lamps and lasers have been used to visualize the cerebral microcirculation. Long exposure periods to fluorescent light should be avoided in order to minimize endothelial cell injury, platelet adhesion and reductions in blood flow. The observation time is typically limited to 30 - 60 seconds for each vessel.

3.3. Ischemia models

Both global and focal ischemia models have been employed to study the cerebromicrovascular responses to I/R. Bilateral common carotid artery occlusion (BCCAO) has been used to induce global ischemia in gerbils (14) and in C57Bl/6 mice (15-18). Uhl (11) observed leukocyte adhesion in microvessels of the gerbil brain after BCCAO and we have performed similar experiments in mice (12). Villringer et al. (19) and Dinagl et al. (20) have used a combination of BCCAO and arterial hypotension to study global ischemia in rats. In the rat, the additional occlusion of the vertebral arteries or the presence of arterial hypotension is necessary to induce global ischemia.

The middle cerebral artery occlusion (MCAO) model, which involves arterial occlusion with an intravascular filament, is the most commonly used model of focal cerebral ischemia. This model was first applied to the rat but it was adapted for the mouse by using a different diameter nylon monofilament. The rat MCAO model was developed in the 1980s (21-23), using 3-0 or 4-0 nylon with a burned tip (0.25~0.3mm) and 15~20mm length from the bifurcation of the internal and external carotid arteries (ICA/ECA) to the origin of the middle cerebral artery (MCA) (24-26). In the mouse MCAO model, a 5-0 or 6-0 nylon thread is used with a burned tip (0.2~0.22mm) and 9~11.5mm length from the ICA/ECA bifurcation to the origin of the MCA (27-32).

A brief summary of the procedure used in our laboratory to produce MCAO in mice follows. After a midline neck incision, the left external carotid artery, the common carotid artery and the pterigoparatine artery are isolated and ligated. The internal carotid artery is clipped at the peripheral site of the bifurcation of the internal carotid artery and the pterigoparatine artery. A tip of a nylon monofilament is blunted with fire or a coagulator. As the suture is advanced to the level of the carotid bifurcation, the nylon and ECA are secured with 5-0 silk suture in order to prevent bleeding during the final occlusion and during the removal of the nylon thread at the time of reperfusion. The ECA is then cut and rotated with the nylon thread to further advance the nylon thread into the ICA. The nylon thread is advanced until a small resistance is felt and until the desired distance from the nylon thread tip to the internal carotid artery-pterygophalatine artery bifurcation and to the ICA-ECA bifurcation are reached. Once the desired ischemic duration is achieved, the nylon thread is removed and the common carotid artery occlusion is opened and reperfused.

Table 1. Experimental studies of leukocyte-endothelial cell interactions after I/R

Ischemia model	Reperfusion time	Species	Main findings	Reference
10min BCCAO & reduction of BP	60min	Rat	Leukocytes were observed with confocal laser scanning microscopy	19
10min BCCAO & reduction of BP	30min, 1h, 2h, 3h, 4h	Rat	Leukocyte plugging is not responsible for the early cortical hypoperfusion.	20
10min asphyxia	30min, 1h, 2h	Newborn piglet	A monoclonal antibody to the CD18 attenuated leukocyte adherence	60
9min asphyxia	1h, 2h	Newborn piglet	PAF mediates ischemia-induced leukocyte adhesion	43
9min asphyxia	1h, 2h	Newborn piglet	Leukocyte adhesion can be prevented by NO.	39.
1h MCAO	30min, 1h, 2h, 3h	Rat	Hypothermia attenuate leukocyte adhesion after I/R	33
60min BCCAO & elevation of ICP to 20mmHg	5min, 60min	Rat	L-NAME augments leukocyte adhesion after I/R	40
9min asphyxia	1h, 2h	Newborn piglet	Hydroxyethyl starch reduces leukocyte adhesion and vascular permeability after I/R	60
1h MCAO	3h	Rat	Glyceol attenuate leukocyte adhesion after I/R	38
2h MCAO	15min, 30min, 1h	Rat	Leukocyte adhesion increased and shear rate decreased after focal I/R	36
15min BCCAO	5min, 20min, 40min, 60min, 1.5h, 2h, 3h, 6h, 7h, 24h, 38h, 72h, 96h	Gerbil	Short lasting activation of leukocytes can play a role in the development of secondary brain damage.	11
1h BCCAO	40min, 4h	mouse	Platelets roll and adhere after I/R	12

I/R, ischemia/reperfusion; BCCAO, bilateral common carotid artery occlusion; MCAO, middle cerebaral artery occlusion; BP, blood pressure; NO, nitric oxide.

We first described the combined use of a cranial window and the MCAO methods in rats (33). With this combination, we can occlude the MCA while observing the brain surface through the cranial window with an intravital fluorescence microscope. When the thread occludes the MCA, sudden retrograde flow is observed in arterial anastomoses, but not in arterioles penetrating into the brain. The same retrograde flow is observed in mice during MCAO. However, it is more difficult to observe the cerebral microvessels in mice via a cranial window during occlusion of the MCA. Since the brain surface in the infarcted area exhibits low flow, not no-flow, some rolling and adherent leukocytes can be seen in venules with slower flow even during MCAO.

4. INFLAMMATORY RESPONSES TO I/R

4.1. Leukocyte adhesion

The leukocyte-endothelial cell interactions elicited in postischemic tissues consists of several steps, i.e., rolling, adhesion and transendothelial migration. The recruited leukocytes can contribute to reperfusion injury through the release of proteases, reactive oxygen species, and other inflammatory mediators. The engagement of adhesion molecules on leukocytes with ligands on endothelial cells can activate signaling pathways in either or both cells, which can lead to further amplification of the inflammatory response. Circulating activated leukocytes can also plug cerebral capillaries and induce capillary no-reflow. We occasionally observe transiently plugging leukocytes within short capillaries of the brain that eventually dislodge and gain access into downstream venules. This plugging of capillaries following I/R has been attributed to the reduced deformability (increased stiffness) of activated leukocytes (34).

4.1.1. Time -course

In 1991, Villringer et al. (19) first demonstrated leukocyte-endothelial cell interactions in the brain microcirculation of the rat after 10 min global ischemia and 60 min reperfusion (Table 1). Later, it was reported that leukocyte rolling and adhesion can be observed as early as 30 min into the reperfusion period after 10 min of global ischemia in rat (20), at 40 min reperfusion after 15 min global ischemia in the gerbil (11) and at 40 min of reperfusion after 60 min global ischemia in the mouse (12). The rapid appearance of rolling and adherent leukocytes in brain venules after I/R are consistent with the rapid upregulation of P-selectin and substantial constitutive expression of ICAM-1 (35) in this vascular bed. Using mutant mice that are genetically deficient in either P-selectin or ICAM-1, we have demonstrated the requirement of these adhesion molecules to sustain the leukocyte rolling and firm adhesion elicited in postischemic cerebral venules (12).

An interesting difference between the leukocyte adhesion responses noted in rats vs mice subjected to MCAO (33,36) is the appearance of adherent leukocytes in arterioles in the former specie, but not the latter. This may relate to the presence of retrograde flow in arterioles in the MCAO model (33,36,37). However, rolling and adherent leukocytes are not observed in arterioles of C57Bl/6 mice even though retrograde flow is observed in these arterioles.

4.1.2. Shear rate

Ritter et al. (36) reported a significant increase in leukocyte adhesion and a significant reduction in venular shear rate after I/R in the brain. Similarly, we have reported that glycerol increases the velocity of flowing leukocytes and reduces leukocyte adhesion in cerebral venules(38). These findings are consistent with studies in other tissues that show a strong inverse relationship between leukocyte adherence and venular shear rate.

4.1.3. Nitric oxide (NO)

The nitric oxide synthase (NOS) inhibitor, L-nitroarginine (L-NA), and the NO donor, sodium nitroprusside (SNP), have been used to demonstrate that NO modulates the leukocyte-endothelial cell adhesion elicited in cerebral venules by asphyxia/reoxygenation (39). Hudetz (40) also demonstrated that NOS inhibitor, N-nitro-L-arginine methyl ester (L-NAME), enhances the leukocyte adhesion induced by I/R while its inactive enantiomer, N-nitro-D-arginine methyl ester (D-NAME), does not. It remains unclear whether the anti-adhesion effects of NO in the cerebral microcirculation are mediated through activation of guanylyl cyclase or it is related to the scavenging of superoxide produced by postischemic tissue.

4.1.4. Platelet activating factor (PAF)

It has been reported that PAF levels are increased in the initial period of reperfusion following ischemia in the brain (41,42). Exogenous PAF has also been shown to induce leukocyte-endothelial cell adhesion in rat cerebral microcirculation (7,8). PAF has also been implicated as a mediator of the leukocyte adhesion observed in the brain following asphyxia/reoxygenation, an effect that appears to be linked to superoxide radical generation (43).

4.1.5.Hypothermia

Hypothermia has been shown to have a neuroprotective influence on brain damage in both the experimental and clinical setting. We first demonstrated using intravital fluorescence microscopy that moderate hypothermia attenuates the leukocyte adhesion in cerebral venules induced by I/R (33). Hypothermia also appears to attenuate ICAM-1 expression.

4.2. Adhesion molecules

Endothelial cell adhesion molecules belonging to the selectin (P-selectin and E-selectin) and the immunoglobulin gene (ICAM-1) superfamilies and their counter-receptors (PSGL-1, CD11/CD18) on leukocytes have been implicated in the pathogenesis of ischemic stroke. The selectins, which mediate leukocyte rolling, and ICAM-1, which mediates the firm adhesion of leukocytes, are expressed on vascular endothelial cells in the postischemic brain (35,44-53). The participation of leukocyte adhesion molecules in ischemic stroke has been examined using monoclonal antibodies directed against either LFA-1 (CD11a), Mac-1 (CD11b), or the common beta subunit, CD18. All of these adhesion molecule neutralizing antibodies reduce postischemic tissue damage in the brain (44,54,55). It has been shown that anti-ICAM-1 or P-selectin or their deficient mice also reductions in the intensity of the inflammatory response, infarct volume, and enhanced functional recovery after cerebral I/R has been demonstrated in wild type mice receiving either ICAM-1 or P-selectin specific antibodies as well as in mice that are genetically deficient in either endothelial cell adhesion molecule (44,47,51,56-59).

In recent years, a number of studies have implicated a role for inflammatory mediators and infiltrating leukocytes in the pathogenesis of cerebral ischemia-reperfusion injury. Evidence derived from animal models of stroke that either employ adhesion molecule-specific antibodies or mutant mice that are genetically deficient in certain adhesion molecules indicate that the adhesion of leukocytes to vascular endothelium is a rate-determining step in the tissue injury and organ dysfunction that occurs after reperfusion of the ischemic brain. Despite this recognition of the potential importance of leukocyte-endothelial cell adhesion in experimental stroke models, there have been few attempts to monitor and quantify leukocyte adhesion in the cerebral microvasculature after I/R. There are a few reports that have demonstrated a role for CD11/CD18 (60), ICAM-1 and P-selectin (12) in mediating the I/R-induced leukocyte adhesion. CD11/CD18 on leukocytes binds to ICAM-1 on endothelial cells, which is important for leukocyte adhesion and P-selectin glycoprotein ligand-1 (PSGL-1) on leukocytes binds to P-selectin on endothelial cells, which is important for leukocyte rolling. Our findings in ICAM-1 and P-selectin deficient mice are consistent with a model that implicates P-selectin as a mediator of leukocyte rolling and ICAM-1 as a mediator of firm adhesion. Since rolling is a prerequisite for firm adhesion, P-selectin deficiency can reduce not only leukocyte rolling and but also firm adhesion, while ICAM-1 deficiency shows no significant effect on leukocyte rolling. Clinically, increased circulating levels of soluble ICAM-1 and P-selectin have been detected in the serum of stroke patients, which is consistent with the view that a portion of these CAMs are shed from the vessel wall after endothelial cell activation.

4.3. Reactive oxygen species

Reactive oxygen species can act as signaling molecules and consequently mediate the cerebral microvascular responses to I/R, including increased adhesion molecule biosynthesis and cell surface expression, increased inflammatory mediator production, and inactivation of NO. Xanthine oxidase, NADPH oxidase, mitochondria respiration, and arachidonic acid have all been implicated as potential sources of ROS in the postischemic brain (61-63).

The first reports of enhanced superoxide production in the brain following I/R were based on the

nitro blue tetrazolium (NBT) method (64) and involved detection by electron microscopy (65). 2,3-and 2.5-dihydroxybenzoic acid (DHBA) as indicators for hydroxyl radicals have also been used with microdialysis methods in the postischemic brain (66,67) along with lucigenin-induced chemiluminescence (68,69). Murakami et al. (63) have proposed that reactive oxygen species from mitochondria play a pivotal role in immediate reperfusion period after ischemia/reperfusion. We have employed the oxidant-sensitive dihydrorhodamine-123 (DHR-123) to visualize and quantify oxidant production in cerebral venules following I/R. Our initial findings indicate that oxidants are produced within one hour after reperfusion.

4.4. Platelet adhesion

Platelets represent a therapeutic target that is gaining attention in both preclinical and clinical studies of stroke. Furthermore, there is new evidence that the inflammatory changes elicited by I/R in the cerebral microcirculation are accompanied by the recruitment of platelets that roll along and firmly adhere to cerebral microvascular endothelium. The recruited platelets appear to amplify the inflammatory response to I/R and may contribute directly to the microvascular dysfunction and tissue injury.

4.4.1. Time-course

Rolling and adherent platelets were first observed in our brain study after I/R (Ishikawa 2003). The rolling and adherent platelets observed in mouse cerebral venules subjected to I/R are not seen until 4 hr after reperfusion, and lags behind the recruitment of rolling and adherent leukocytes, which is manifested as early as 40 min after reperfusion. Platelet-vessel wall interactions have been already described in intestine (70,71), retina (72) and liver (73) after ischemia-reperfusion. In mouse small bowel exposed to I/R, platelet adhesion was noted in both arterioles and venules as early as 5 min after reperfusion in Massberg's report, but it was observed just in venules 4 hr after reperfusion in Cooper's report. In mouse liver, platelet adhesion was also observed in both arterioles and venules as early as 5 min after reperfusion. In rat retina (72) and brain (12), the recruitment of rolling and adherent platelets in venules (not arterioles) is not seen until 4 hr after reperfusion. Hence in the brain, eye and some intestine models, platelet recruitment in venules appears to involve a transcription-dependent mechanism and seems to require sufficient time for accumulation of specific mediators of platelet adhesion.

4.4.2. Adhesion molecules for platelet rolling and adhesion

In our study (12) using ICAM-1 deficient mice and P-selectin deficient mice, we demonstrated that ICAM-1 and P-selectin are important for platelet rolling and adhesion. When P-selectin deficient platelets were infused into the postischemic mouse and platelets from WT mice were infused into the postischemic P-selectin deficient mouse, rolling and adherence of platelets were attenuated significantly, compared with the responses seen when platelets from WT platelets were infused into WT mouse after I/R. These findings implicate both platelet- and endothelial cell-associated P-selectin in the platelet accumulation in venules of the postischemic brain.

When platelets from WT mice were infused into the ICAM-1 deficient mouse after I/R, platelet rolling and adhesion are also attenuated, compared with the responses noted when platelets from WT platelets were infused into a WT mouse after I/R. Although there is no known ligand for ICAM-1 on platelets, these findings implicate ICAM-1 in I/R-induced platelet adhesion in cerebral venules. In a published report on platelet adhesion in postischemic mouse intestine, it was shown that ICAM-1-/- mice exhibited an attenuation of platelet adhesion of a magnitude comparable to that seen in our brain experiments. This observation, coupled to additional data related to fibrinogen deposition in the postischemic intestinal vasculature of WT and ICAM-1-/- mice, led these authors to propose that ICAM-1 bound fibrinogen on endothelial cells serves as a ligand for GPIIb/IIIa on platelets. The protective effects of ICAM-1 deficiency against cerebral I/R-induced platelet recruitment noted in our study may be explained by a comparable mechanism. Another model that can explain the findings would involve endothelial P-selectin and ICAM-1 as mediators of the rolling and firm adhesion (respectively) of leukocytes in postischemic venules and the adherent leukocytes then create a platform onto which platelets can bind using P-selectin, because leukocytes (but not endothelial cells) express PSGL-1 which is also a ligand for P-selectin on platelets. Irrespective of the precise mechanism involved in this platelet recruitment process, the available data indicate that the increased expression of P-selectin on both platelets and endothelial cells is of immense quantitative importance in mediating the platelet-vessel wall interactions elicited by cerebral I/R.

5. SUMMARY

Leukocyte- and platelet- endothelial cell interactions have been described in postcapillary venules of the brain exposed to either global or focal ischemia, followed by reperfusion. These interactions are mediated by a variety of factors, including adhesion molecules (eg. P-selectin and ICAM-1), shear rate, NO, and PAF. While there is a large body of evidence that implicates leukocyte adhesion in the pathogenesis of stroke the nature of the contribution of leukocytes to this injury process remains poorly understood. Additional research is needed to define the mechanisms that underlie the recruitment and activation of both leukocytes and platelets in the ischemic and postischemic brain. This effort may lead to improved therapeutic strategies for management of the stroke patient.

6. REFERENCES

1. Del Zoppo, G.J. & Garcia, J.H. Plymorphonuclear leukocyte adhesion in cerebravascular ischemia: pathophysiologic implications of leukocyte adhesion. In: Physiology and pathophysiology of leukocyte adhesion. Eds: Granger DN, Schmid-Schoenbein GW. Oxford University Press, NY 408-425 (1995).

2. Hallenbeck, J.M. Dutka, A.J. Tanishima, T. Kochanek, P.M. Kumaroo, K.K. Thompson, C.B. Obrenovitch, T.P. & T.J. Contreras: Polymorphonuclear leukocyte accumulation in brain regions with low blood flow during the early postischemic period. *Stroke* 17, 246-253 (1986)

3. Schuerer, L. Corvin, S. Roehrich, F. Abels, C. & A. Baethmann: Leukocyte/endothelial interactions and blood-brain barrier permeability in rats during cerebral superfusion with LTB4. *Acta Neurochir suppl* 60, 51-54 (1994)

4. Lindauer, U. Dreier, J. Angstwurm, K. Rubin, I. Villringer, A. Einhaeupl, K.M. & U. Dirnagl: Role of nitric oxide synthase inhibition in leukocyte-endothelium interaction in the rat pial microvasculature. *J Cereb Blood Flow Metab* 16, 1143-1152 (1996)

5. Weber, J.R. Angstwurm, K. Rosenkranz, T. Lindauer, U. Freyer, D. Buerger, W. Busch, C. Einhaeupl, K.M. & U. Dirnagl: Heparin inhibits leukocyte rolling in pial vessels and attenuates inflammatory changes in a rat model of experimental bacterial meningitis. *J Cereb Blood Flow Metab* 17, 1221-1229 (1997)

6. Haertl, R. Medary, M.B. Ruge, M. Arfors, K.E. Ghahremani, F. & J. Ghajar: Hypertonic/hyperoncotic saline attenuates microcirculatory disturbances after traumatic brain injury. *J Trauma* 42, S41-S47 (1997)

7. Uhl, E. Pickelmann, S. Roehrich, F. Baethmann, A. & L. Schuerer: Influence of platelet-activating factor on cerebral microcirculation in rats. Part 1. systemic application. *Stroke* 30, 873-879 (1999)

8. Uhl, E. Pickelmann, S. Roehrich, F. Baethmann, A. and L. Schuerer: Influence of platelet-activating factor on cerebral microcirculation in rats. Part 2. Local application. *Stroke* 30, 880-886 (1999)

9. Carvalho-Tavares, J. Hickey, M.J. Hutchison, J. Michaud, J. Sutcliffe, I.T. & P. Kubes: A role for platelets and endothelial selectins in tumor necrosis factor-alpha-induced leukocyte recruitment in the brain microvasculature. *Circ Res* 87, 1141-1148 (2000)

10. Morii, S. Ngai, A.C. & H.R. Winn: Reactivity of rat pial arterioles and venules to adenosine and carbon dioxide: with detailed description of the closed cranial window technique in rats. *J Cereb Blood Flow Metab* 6, 34-41 (1986)

11. Uhl, E. Beck, J. Stummer, W. Lehmberg, J. & A. Baethmann: Leukocyte-endothelium interactions in pial venules during the early and late reperfusion period after global cerebral ischemia in gerbils. *J Cereb Blood Flow Metab.* 20, 979-87 (2000)

12. Ishikawa, M. Cooper, D. Russell, J. Salter, J.W. Zhang, J.H. Nanda, A. & D.N. Granger: Molecular determinants of the prothrombogenic and inflammatory phenotype assumed by the postischemic cerebral microcirculation. *Stroke* 34, 1777-1782 (2003)

13. Baatz, H. Steinbauer, M. Harris, A.G. & F. Krombach: Kinetics of white blood cell staining by intravascular administration of rhodamine 6G. *Int J Microcirc Clin Exp* 15, 85-91 (1995)

14. Jarott, D.M. & F.R. Comer: A gerbil model of cerebral ischemia suitable for drug evaluation. *Stroke* 11, 203-209 (1980)

15. Fujii, M. Hara, H. Meng, W. Vonsattel, J.P. Huang, Z. & M.A. Moskowitz: Strain-related differences in susceptibility to transient forebrain ischemia in SV-129 and C57black/6 mice. *Stroke.* 28, 1805-1810 (1997)

16. Yang, G. Kitagawa, K. Matsushita, K. Mabuchi, T. Yagita, Y. Yanagihara, T. & M. Matsumoto: C57BL/6 strain is most susceptible to cerebral ischemia following bilateral common carotid occlusion among seven mouse strains: selective neuronal death in the murine transient forebrain ischemia. *Brain Res.* 752, 209-218 (1997)

17. Kitagawa, K. Matsumoto, M. Yang, G. Mabuchi, T. Yagita, Y. Hori, M. & T. Yanagihara: Cerebral ischemia after bilateral carotid artery occlusion and intraluminal suture occlusion in mice: evaluation of the patency of the posterior communicating artery. *J Cereb Blood Flow Metab.* 18, 570-9 (1998)

18. Terashima, T. Namura, S. Hoshimaru, M. Uemura, Y. Kikuchi, H. & N. Hashimoto: Consistent injury in the striatum of C57BL/6 mice after transient bilateral common carotid artery occlusion. *Neurosurgery.* 43, 900-7 (1998)

19. Villringer, A. Dirnagl, U. Them, A. Schuerer, L. Krombach, F. & K.M. Einhaeupl: Imaging of leukocytes within the rat brain cortex in vivo. *Microvasc Res* 42, 305-315 (1991)

20. Dirnagl, U. Niwa, K. Sixt , G. & A. Villringer: Cortical hypoperfusion after global forebrain ischemia in rats is not caused by microvascular leukocyte plugging. *Stroke* 25, 1028-1038 (1994)

21. Koizumi J, Yoshida Y, Nakazawa T, Ooneda G. Experimental studies of ischemic brain edema. 1. A new experimental model of cerebral embolism in rats in which recirculation can be introduced in the ischemic area. Jpn J Stroke 8:1-8 (1986)

22. Longa, E.Z. Weinstein, P.R. Carlson, S. & R. Cummins: Reversible middle cerebral artery occlusion without craniectomy in rats. *Stroke* 20, 84-91 (1989)

23. Nagasawa, H. & K. Kogure: Correlation between cerebral blood flow and histologic changes in a new rat model of middle cerebral artery occlusion. *Stroke* 20, 1037-1043 (1989)

24. Laing, R.J. Jakubowski, J. & R.W. Laing: Middle cerebral artery occlusion without craniectomy in rats. Which method works best? *Stroke* 24, 1143-1152 (1993)

25. Belayev, L. Alonso, O.F. Busto, R. Zhao, W. & M.D. Ginsberg: Middle cerebral artery occlusion in the rat by

intraluminal suture Neurological and pathological evaluation of an improved model. *Stroke* 27, 1616-1623 (1996)

26. Oezdemir, Y.G. Bolay, H. Erdem, E. & T. Dalkara: Occlusion ot the MCA by an intraluminal filament may cause disturbances in the hippocampal blood flow due to anomalies of circle of Willis and filament thickness. *Brain Res* 822, 260-264 (1999)

27. Kamii, H. Kinouch, H. Sharp, F.R. Epstein, C.J. Sagar, S.M. & Chan, P.H: Expression of c-fos mRNA after a mild focal cerebral ischemia in SOD-1 transgenic mice. *Brain Res* 662, 240-244 (1994)

28. Huang, Z. Huang, P.L. Ma, J. Meng, W. Ayata, C. Fishman, M.C. & M.A. Moskowitz: Enlarged infarcts in endothelial nitric oxide synthase knockout mice are attenuated by Nitro-L- Arginine. *J Cerebral Blood Flow Metab* 16, 981-987 (1996)

29. Clark, W.M. Lessov, N.S. Dixon, M.P. & F. Eckenstein: Monofilament intraluminal middle cerebral artery occlusion in the mouse. *Neurol Res* 19, 641-648 (1997)

30. Hata, R. Mies, G. Wiessner, C. Fritze, K. Hasselbarth, D. Brinker, G. & K.A. Hassmann: A reproducible model of middle cerebral artery occlusion in mice: hemodynamic, biochemical, and magnetic resonance imaging. *J Cereb Blood Flow Metab* 18, 367-375 (1998)

31. Belayev, L. Busto, R. Zhao, W. Fernandez, G. & M.D. Ginsberg: Middle cerebral artery occlusion in the mouse by intraluminal suture coated with poly-L-lysine: neurological and histological validation. *Brain Research* 833, 181-190 (1999)

32. Hata, R. Maeda, K. Hermann, D. Mies, G. & K.A. Hossmann: Evolution of brain infarction after transient focal cerebral ischemia in mice. *J Cereb Blood Flow Metab* 20, 937-946 (2000)

33. Ishikawa, M. Sekizuka, E. Sato, S. Yamaguchi, N. Inamasu, J. Bertalanffy, H. & T. Kawase: Effects of moderate hypothermia on leukocyte- endothelium interaction in the rat pial microvasculature after transient middle cerebral artery occlusion. *Stroke*. 30, 1679-86 (1999)

34. M.D. Menger: Molecular determinants of reperfusion-induced leukocyte adhesion. In: Molecular basis for microcirculatory disorders. Eds: Schmid-Schoenbein GW, Granger DN. Springer-Verlag France, Paris 315-332 (2003).

35. Okada, Y. Copeland, B.R. Mori, E. Tung, M.M. Thomas, W.S. & G.J. del Zoppo: P-selectin and intercellular adhesion molecule-1 expression after focal brain ischemia and reperfusion. *Stroke* 25, 202-211 (1994)

36. Ritter, L.S. Orozco, J.A. Coull, B.M. McDonagh, P.F. & W.I. Rosenblum: Leukocyte accumulation and hemodynamic changes in the cerebral microcirculation during early reperfusion after stroke. *Stroke.* 31, 1153-61 (2000)

37. Nazziola, E. & S.D. House: Effects of hydrodynamics and leukocyte-endothehelium specificity on leukocyte-endothelium interactions. *Microvasc Res* 44, 127-142 (1992)

38. Ishikawa, M. Sekizuka, E. Sato, S. Yamaguchi, N. Inamasu, J. & T. Kawase: Glycerol attenuates the adherence of leukocytes in rat pial venules after transient middle cerebral artery occlusion. *Neurol Res* 21, 785-790 (1999)

39. Gidday, J.M. Park, T.S. Shah, A.R. & E.R. Gonzales: Modulation of basal and postischemic leukocyte-endothelial adherence by nitric oxide. *Stroke* 29, 1423-1430 (1998)

40. Hudetz, A.G. Wood, J.D. & J.P. Kampine: Nitric oxide synthase inhibitor augments post-ischemic leukocyte adhesion in the cerebral microcirculation in vivo. *Neurol Res* 21, 378-384 (1999)

41. Domingo, M.T. Spinnewyn, B. Chabrier, P.E. & P. Braquet: Changes in [3H]PAF binding and PAF concentrations in gerbil brain after bilateral common carotid artery occlusion: a quantitative autoradiographic study. *Brain Res* 640, 268-276 (1994)

42. Nishida, K. & S.P. Markey: Platelet-activating factor in brain regions after transient ischemia in gerbil. *Stroke* 27, 514-519 (1996)

43. Park, T.S. Gonzales, E.R. & J.M. Gidday: Platelet-activating factor mediates ischemia-induced leukocyte-endothelial adherence in newborn pig brain. *J Cereb Blood Flow Metab* 19, 417-424 (1999)

44. Matsuo, Y. Onodera, H. Shiga, Y. Shozuhara, H. Ninomiya, M. Kihara, T. Tamatani, T,. Miyasaka, M. & K. Kogure: Role of cell adhesion molecules in brain injury after transient middle cerebral artery occlusion in the rat. *Brain Res* 656, 344-352 (1994)

45. Hess, D.C. Zhao, W. Carroll, J. McEachin, M. & K. Buchanan: Increased expression of ICAM-1 during reoxygenation in brain endothelial cells. *Stroke* 25:1463-1468 (1994)

46. Wang, X. Siren, A.L. Liu, Y. Yue, T.L. Barone, F.C. & G.Z. Feuerstein: Upregulation of intercellular adhesion molecule 1 (ICAM-1) on brain microvascular endothelial cells in rat ischemic cortex. *Mol Brain Res* 26, 61-68 (1994)

47. Zhang, R.L. Chopp, M. Li, Y. Zaloga, C. Jiang, N. Jones, M.L. Miyasaka, M. & P.A. Ward: Anti-ICAM-1 antibody reduces ischemic cell damage after transient middle cerebral artery occlusion in the rat. *Neurology* 44, 1747-1751 (1994)

48. Wang, X. Yue, T.L. Barone, F.C. & G.Z. Feuerstein: Demonstration of increased endothelial-leukocyte adhesion molecule-1 mRNA expression in rat ischemic cortex. *Stroke* 26, 1665-1669 (1995)

49. Zhang, R.L. Chopp. M. Jiang, N. Tang, W.X. Prostak, J. Manning, A.M. & D.C. Anderson: Anti-intercelular adhesion molecule-1 antibody reduces ischemic cell damage after transient but not permanent middle cerebral artery occlusion in the wistar rat. *Stroke* 26, 1438-1443 (1995)

50. Haring, H.P. Berg, E.L. Tsurushita, N. Tagaya, M. & G.J. del Zoppo: E-selectin appears in nonischemic tissue during experimental focal cerebral ischemia. *Stroke* 27, 1386-1392 (1996)

51. Connolly, E.S. Winfree, C.J. Prestigiacomo, C.J. Choudhri, T.F. Hoh, B.L. Naka, Y. Solomon, R.A. & D.J. Pinsky: Exacerbation of cerebral injury in mice that express the P-selectin gene. *Circ Res* 81, 304-310 (1997)

52. Suzuki, H. Abe, K. Tojo, S. Morooka, S. Kimura, K. Mizugaki, M. & Y. Itoyama: Postischemic expression of P-selectin immunoreactivity in rat brain. *Neurosci Lett* 228, 151-154 (1997)

53. Zhang, R. Chopp, M. Zhang, Z. Jiang, N. & C. Powers: The expression of P- and E-selectins in three modles of middle cerebral artery occlusion. *Brain Res* 785, 207-214 (1998)

54. Chen, H. Chopp, M. Zhang, R.L. Bodzin, G. Chen, Q. Rusche, J.R. & R.F. Todd III: Anti-CD11b monoclonal antibody reduces ischemic cell damage after transient focal cerebral ischemia in rat. *Ann Neurol* 35, 458-463 (1994)

55. Chopp, M. Zhang, R.L. Chen, H. Li, Y. Jiang, N. & J.R. Rusche: Postischemic administration of an anti-Mac-1 antibody reduces ischemic cell damage after transient middle cerebral artery occlusion in rats. *Stroke* 25, 869-876 (1994)

56. Zhang, R.L. Chopp, M. Zaloga, C. Zhang, Z.G. Jiang, N. Gautam, S.C. Tang, W.X. Tsang, W. Anderson, D.C. & A.M. Manning: The temporal profiles of ICAM-1 protein and mRNA expression after transient MCA occlusion in the rat. *Brain Res* 682, 182-188 (1995)

57. Soriano, S.G. Lipton, S.A. Wang, Y.F. Xiao, M. Springer, T.A. Gutierrez-Romos, J.C. & P.R. Hickey: Intercellular adhesion molecule-1-deficient mice are less susceptible to cerebral ischemia-reperfusion injury. *Ann Neurol* 39, 618-624 (1996)

58. Kitagawa, K. Matsumoto, M. Mabuchi, T. Yagita, Y. Ohtsuki, T. Hori, M. & T. Yanagihara: Deficiency of intercellular adhesion molecule 1 attenuates microcirculatory disturbance and infarction size I focal cerebral ischemia. *J Cereb Blood Flow Metab* 18, 1336-1345 (1998)

59. Suzuki, H. Abe, K. Tojo, S.J. Kitagawa, H. Kimura, K. Mizugaki, M. & Y. Itoyama: Reduction of ischemic brain injury by anti-P-selectin monoclonal antibody after permanent middle cerebral artery occlusion in rat. *Neurol Res* 21, 269-276 (1999)

60. Gidday, J.M. Park, T.S. Gonzales, E.R. & J.W. Beetsch: CD18-dependent leukocyte adherence and vascular injury in pig cerebral circulation after ischemia. *Am J Physiol.* 272, H2622-9 (1997)

61. Matsuo, Y. Kihara, T. Ikeda, M. Ninomiya, M. Onodera, H. & K. Kogure: Role of neutrophils in radical production during ischemia and reperfusion of the rat brain: effect of neutrophil depletion on extracellular ascorbyl radical formation. *J Cereb Blood Flow Metab* 15, 941-947 (1995)

62. Walder, C.E. Green, S.P. Darbonne, W.C. Mathias, J. Rae, J. Dianauer, M.C. Curnutte, J.T. & G.R. Thomas: Ischemic stroke injury is reduced in mice lacking a functional NADPH oxidase. *Stroke* 28, 2252-2258 (1997)

63. Murakami, K. Kondo, T. Kawase, M. Li, Y. Sato, S. Chen, S.F. & P.H. Chan: Mitochondrial susceptibility to oxidative stress exacerbates cerebral infarction that follows permanent focal cerebral ischemia in mutant mice with manganese superoxide dismutase dificiency. *J Neurosci* 18, 205-213 (1998)

64. Nelson, C.W. Wei, E.P. Povlishock, J.T. Kontos, H.A. & M.A. Moskowitz: Oxygen radicals in cerebral ischemia. *Am J Physiol* 263, H1356-H1362 (1992)

65. Kontos, C.D. Wei, E.P. Williams, J.I. Kontos, H.A. & J.T. Povlischock: Cytochemical detection of superoxide in cerebral inflammation and ischemia in vivo. *Am J Physiol* 263, H1234-1242 (1992)

66. Kil, H.Y. Zhang, J. & C.A. Piantadosi: Brain temperature alters hydroxyl radical production during cerebral ischemia/reperfusion in rats. *J Cereb Blood Flow Metab* 16, 100-106 (1996)

67. Morimoto, T. Globus, M.Y.T. Busto, R. Martinez, E. M.D. Ginsberg: Simultaneous measurement of salicylate hydrosylation and glutamate release in the penumbral cortex following transient middle cerebral artery occlusion in rats. *J Cereb Blood Flow Metab* 16, 92-99 (1996)

68. Dirnagl, U. Lindauer, U. Schreiber, S. Pfister, H.W. Koedel, U. Reszka, R. Freyer, D. & A. Villringer: Global cerebral ischemia in the rat: online monitoring of oxygen free radical production using chemiluminescence in vivo. *J Cereb Blood Flow Metab* 15, 929-940 (1995)

69. Peters, O. Back, T. Lindauer, U. Busch, C. Megow, D. Dreier, J. & U. Dirnagl: Increased formation of reactive oxygen species after permanent and reversible middle cerebral artery occlusion in the rat. *J Cereb Blood Flow Metab* 18, 196-205 (1998)

70. Massberg, S. Enders, G. Leiderer, R. Eisenmenger, S. Vestweber, D. Krombach, F. & K. Messmer: Platelet-endothelial cell interactions during ischemia/reperfusion: the role of P-selectin. *Blood.* 92, 507-15 (1998)

71. Cooper, D. Chitman, K.D. Williams, M.C. & D.N. Granger: Time-dependent platelet-vessel wall interactions induced by intestinal ischemia-reperfusion. *Am J Physiol Gastrointest Liver Physiol* 284, G1027-G1033 (2003)

72. Nishijima, K. Kiryu, J. Tsujikawa, A. Honjo, M. Nonaka, A. Yamashiro, K. Tanihara, H. Tojo, S.J. Ogura, Y. & Y. Honda: In vivo evaluation of platelet--endothelial interactions after transient retinal ischemia. *Invest Ophthalmol Vis Sci.* 42, 2102-2109 (2001)

73. Khandoga, A. Biberthaler, P. Enders, G. Axmann, S. Hutter, J. Messmer, K. & F. Krombach: Platelet adhesion mediated by fibrinogen-intercellular adhesion molecule-1 binding induces tissue injury in the postischemic lever in vivo. *Transplatation* 74, 681-688 (2002)

Key Words: Nervous system, Brain, Vessel, Leukocyte, Inflammation, Cerebral, Ischemia, Fluorescence Microscopy, Rewview

Send correspondence to: D. Neil Granger, PhD, Department of Molecular and Cellular Physiology, Louisiana State University Health Sciences Center, 1501 Kings Highway, Shreveport Louisiana 71130-3932, Tel.: 318 675 6011, Fax: 318 675 6005, Email: dgrang@lsuhsc.edu