Adhesion Molecules in Cerebral Ischemia and Atherosclerosis

Nureddin Cengiz[1], Ender Erdoğan[1], Hanefi Özbek[2], Mustafa Tuncer[3]

Yüzüncü Yıl University, Medical Faculty, Departments of Histology[1], Pharmacology[2] and Cardiology

Eur J Gen Med 2009; 6(4): 249-256

ABSTRACT

Adhesion molecules are integral membrane proteins that have cytoplasmic, transmembrane and extracellular domains. Dozens of different adhesion molecules have been identified, but, adhesion molecules are conventionally divided into four main groups, each of which has a different function:

1. The selectins,

2. The immunglobulin gene superfamily,

3. The integrins and

4. The cadherins.

Under normal conditions, there is little or no cell-surface expression of adhesion molecules. Various inflammatory processes induce their expressions, such as atherosclerosis and cerebral ischemia, with the upregulations mediated by cytokines. Normally, vascular endothelial cells have low adhesiveness for leukocytes; however, when stimulated they express adhesion molecules at their surfaces responsible for adhesion and activation of leukocytes as a precondition for transendothelial migration of leukocytes. The effect of anti-adhesion molecule strategies in focal cerebral and spinal cord ischemia showed a beneficial effect in models in which transient focal ischemia was followed by reperfusion, but not in models of permanent ischemia. In addition anti-inflammatory agents could have reduced expression and shedding of adhesion molecules as a result of its antiinflammatory properties.

Key words: *Atherosclerosis, cerebral ischemia, adhesion molecules.*

Correspondence:
Nureddin Cengiz
Yüzüncü Yıl Üniversitesi
Tıp Fakültesi Histoloji ve Embriyoloji Anabilim Dalı
Temel Bilimler Binası, Van, Türkiye
E-mail: nurettincengiz@hotmail.com

INTRODUCTION

Adhesion molecules are glycoprotein molecules, expresses on the surfaces of cells that assist cells in interacting with their environments through adherence.

All adhesion molecules are integral membrane proteins that have cytoplasmic, transmembrane and extracellular domains. The cytoplasmic tail often interacts with cytoskeletal proteins, which serve as the actual anchor within the cell. The extracellular domains of adhesion molecules extend from the cell and bind to other cells or matrix by binding to other adhesion molecules of the same type (homophilic binding), binding to other adhesion molecules of a different type (heterophilic binding) or binding to an intermediary "linker" which itself binds to other adhesion molecules.

Most cells are decorated with several types of proteins that allow their binding to other cells or to the extracellular matrix. The list of important functions of adhesion molecules is a long one, but some of their most follow:

- Tissue, organ development and cell proliferation,
- Reproductive physiology
- Embryogenesis
- Immune and inflammatory cell migration
- Starting and expanding immune response
- Interacting between cell and extracellular matrix
- Wound healing
- Cancer metastasis (1-6).

Also inflammatory leukocyte-endothelial adhesion molecules are a play role in the ischemic cerebrovascular pathogenesis. It effects by their receptors. Dozens of different adhesion molecules have been identified, but, adhesion molecules are conventionally divided into four main groups, each of which has a different function:

1. The selectins,
2. The immunglobulin gene superfamily,
3. The integrins and
4. The cadherins.

Under normal conditions, there is little or no cell-surface expression of adhesion molecules. Various inflammatory processes induce their expressions, such as cerebral ischemia, with the up regulations mediated by cytokines (7).

Selectins mediate rolling of leukocytes on the endothelium of post capillary venules. E selectin (CD62E) is synthesized after stimulation by cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1) and expressed on the endothelial cell membrane after several hours. P selectin (CD62P) is constitutively present on granule membranes in endothelial cells and platelets. Therefore, it can be expressed on the outer cell membrane immediately on cell activation by stimulants such as thrombin or histamine. The target cells of both E- and Pselectin are neutrophils and monocytes. Counterreceptors on these white blood cells are carbohydrate structures on membrane glycoproteins and L-selectin. L-selectin (CD62L) is present on lymphocytes, neutrophils, and monocytes. After cellular activation, it is shed from the cell membrane by proteolytic cleavage (8, 9, 10).

Firm adhesion of leukocytes to the endothelial cells as well as leukocyte activation is mediated by receptors of the immunoglobulin gene superfamily. To this family belong 5 molecules that are expressed by endothelial cells: Intercellular adhesion molecule-1 and -2 (ICAM-1 and ICAM-2), vascular cell adhesion molecule-1 (VCAM-1), platelet-endothelial cell adhesion molecule-1 (PECAM- 1), and the mucosal addressin (MAdCAM-1). The CAMs are involved in leukocyte adhesion at relatively low shear forces; they cause a stronger attachment of leukocytes to the endothelium than the selectins (8). ICAM-1 (CD54), a single chain 76-110 kDa glicoprotein, has been identified as a ligand for leukocyte adhesion molecule designated as a lymphocyte function-associated antigen (LFA-1). The interaction of ICAM-1 with LFA-1 on the endothelial cells is thought to facilitate lymphocyte migration and infiltration of inflammatory cells (11). ICAM-1 is continuously present in low amounts on the cell membranes of endothelial cells, leukocytes, epithelial cells, and fibroblasts. Its expression greatly increases on stimulation by cytokines. ICAM-2 (CD102) is a membrane receptor of endothelial cells that does not increase after stimulation, whereas VCAM-1 (CD106) expression on endothelial cells is induced by TNF-α and IL-1. PECAM-1 (CD31) has a role in the attachment of endothelial cells to each other, in leukocyte adhesion, and particularly in transmigration across the endothelium. Its surface expression on endothelial cells is not increased by cytokines (8).

After rolling of the leukocyte on the endothelial surface has arrested its flow, leukocyte integrins are

activated by chemokines, chemoattractants and cytokines. Integrins are heterodimeric membrane glycoproteins formed by the combination of an á- and â-subunit with an intracellular and extracellular domain (7). The CD18 or ß2 integrins are restricted to leukocytes and bind to their counterreceptors of the immunoglobulin gene superfamily (8). They share a common ß chain and 3 distinct á chains (CD11a, CD11b, or CD11c). Their surface expression is increased by agonists such as TNF-α and after adhesion to E-selectin. Leukocyte integrins are involved in the firm adherence of the leukocyte through binding to the endothelial Ig gene superfamily molecules (8,9,10,12). Leukocytes and monocytes also express the integrin α4ß1 (very late antigen-4 [VLA-4], CD49d), which binds to VCAM-1 and to ligands from the subendothelial matrix (8). At the basal membrane level integrins connect endothelial cells to extracellular components such as laminin and collagen. In the brain, endothelial cells, astrocytes and the basal membrane contribute to form the blood-brain barrier, and their interconnection is mediated by integrins. Damage to these molecules may lead to severe damage of the blood-brain barrier (7). Integrin α6β4, mediates the inteaction between astrocytes and extracellular matrix, is rapidly damaged during focal cerebral ischemia/ reperfusion. Other inttegrins play an important role in inflammatory neoangiogenesis, wound repair and ontogenesis and may therefore be important for tissue repair after an ischemic insult (7,13).

Cadherins are cell adhesion molecules originally identified as a cell surface molecule responsible for Ca21- dependent cell adhesion (14). The homophilic interaction of cadherin confers cell-cell binding interaction and adhesion specificity on cells that relate to segregation, morphogenesis, neural network formation, and tumor metastasis (14). Early characterization and molecular cloning revealed the presence of three distinct cadherin molecules E-, 1 N-, and P cadherin, in which their cell and tissue specifity and temporal expression are quite different. Cadherins are transmembrane proteins consisting of an extracellular domain that confers homophilic Ca21- dependent cell-cell binding, a transmembrane domain, and a cytoplasmic domain. The extracellular domain contains five cadherin repeat motifs and mediates calcium-dependent cell-cell interaction. The cytoplasmic domain of cadherin interacts with intracellular proteins, a-, b-, and g-catenins (14). a-Catenin interacts with cytoskeletal proteins, whereas b-catenin is considered to regulate the function of cell-cell adhesion by tyrosine phosphorylation. In addition to classical E-, N-, and P-cadherins, recent work revealed that cadherin-related molecules are structurally diverse and that they constitute a cadherin superfamily. R-cadherin, B-cadherin, OB-cadherin, and cadherin 4-11 conserve a membrane spanning structure in classic cadherins. In contrast, T-cadherin lacks both the transmembrane domain and the conserved cytoplasmic domain but is attached to the plasma membrane anchored with a glycosyl phosphatidylinositol. Protocadherins contain 6 or 7 extracellular repeats of the cadherin motif and the cytoplasmic domain not homologous to that of other cadherins. Desmogleins, pemphigus vulgaris antigen, and desmocollins were identified as the adhesion molecule localized at the desmosome (14).

Ischemic cell damage after MCA occlusion may be mediated by many factors, among which are inflammatory processes (15-17). An inflammatory reaction is a common response of the brain parenchyma to various forms of insult. It is histologically characterized by the infiltrations of leukocytes and monocytes/ macrophages (7,18).

Experimental studies in animal models of focal ischemic stroke have suggested that polymorphonucleer leukocytes play a role in the development of secondary injury after acute ischemic infarction (8, 19). An increased number of polymorphonuclear leukocytes in the microvessels or ischemic brain parenchyma have been found in various species (7,20-22). In experimental models of stroke, peripheral blood leokocytes migrate into the brain parenchyma within the first 12 hours after ischemia (23,24). The appearance of leukocytes in injured ischemic tissue is not only a pathophysiological response to existing injury but also may promote ischemic injury (15,25).

The adhesion of leukocytes to the endothelial surface and their subsequent migration from the microvessels into the brain parenchyma is mediated by a variety of molecules located on the surfaces of both leukocytes and endothelial cells (7,26). Normally, vascular endothelial cells have low adhesiveness for leukocytes; however, when stimulated they express adhesion molecules at their surfaces responsible for adhesion and activation of leukocytes as a precondition for transendothelial migration of leukocytes (4,27).

The leukocyte-endothelial adhesion processes consist of several steps, beginning with rolling of the leukocytes on the endothelial surface until it has slowed down to such a degree that it sticks to the endothelium. At this point the leukocyte becomes activated and flattens. Firm adherence to the endothelial cells follows this step. Subsequently, the leukocyte crawls on the endothelium to find an intercellular junction between the endothelial cells for diapedesis to the abluminal side and for transmigration to the target tissue (8,28).

The signals mediating the entry of leukocytes into the ischemic area have not yet been completely elucidated, but a number clue suggests that cytokines play a key role in this process. Cytokines are low molecular weight glicoproteins that act as intercellular messengers and are produced by macrophages, monocytes, lymphocytes, endothelial cells, fibroblasts, platelets, astrocytes, neurons and many other cells. All of these cells need to be activated to produce cytokines (7,29,30). Cytokines are considered to be among the principal mediatorsof immunologic and inflammatory responses that may have various functions during cerebral ischemia: on the one hand, they can attract leokocytes and stimulate the synthesis of adhesion molecules in leukocytes, endothelial cells and other cells, thus promoting the inflammatory response of damaged cerebral tissue, on the other they can facilitate thrombogenesis by increasing the levels of plasminogenactivating inhibitor-1, tissue factor and platelet activating factor (7,31).

Endothelial-leukocyte adhesion molecule (E-selectin) binds to an overlapping set of carbohydrate structures at leukocyte surfaces, whereas the members of the immunoglobulin gene superfamily of adhesion molecules, vascular cell adhesion molecules-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1), interact with integrins at cellular surfaces (4,27). In contrast to ICAM- 1, which can also be expressed on leukocytes, fibroblasts or epithelial cells, E-selectin and VCAM-1 are exclusively expressed on endothelial cells (4). Other factors currently thought to contribute to endothelial activation are hemodynamic shear stress (4,32), excessive load with low-density lipoprotein (4,33), or chronic infections of the vasculature (4,34,35). These noxious stimuli could activate endothelial cells, either directly or indirectly via stimulation of local mononuclear phagocytes as principal producers of proinflammatory cytokines involved in upregulation of endothelial adhesion molecules (4,27) In small arteries, arterioles and capillaries, adherence of leukocytes and their transendothelial migration mediated by ICAM-1 or Eselectin may be responsible for vascular injury by release of reaktive oxygen metabolites, granular enzimes or toxic or growth-promoting cytokines (27,36).

There are a number of mechanisms by which leukocytes may produce deleterious effects on ischemic parenchima. It has been proposed that leukocytes obstruct the microvessels by adhere to the activated endothelium and contribute toward the so-called "no-reflow" phenomenon (7,23,37); i.e, the lack of complete recovery of cerebral blood flow in the ischemic area after reperfusion (7). Reperfusion of the ischemic tissue leads to rapid accumulation of neutrophils (38). Acute leukocyte microvascular occlusion and leukocytes infiltration into ischemic area potentiate ischemic cell damage (15). This plugging of microvessels under ischemic conditions may be the result of an interaction with endothelial cells, mediated by adhesion molecules and/or the loss of cell deformability due to actin polimerization and pseudopod projections (15,39). As reviewed by Hartl et al (40) other detrimental effects of leukocytes during ischemia may be due to:

1. The release of vasoconstructive mediators, such as superoxide anions, thromboxane A2, endothelin-1 and prostaglandin H2 (7,41),
2. An alteration in cerebral artery vasoreactivity and
3. The release of cytotoxic enzymes, free oxygen radicals, NO, and products of the phospholipid cascade.

The release of proteolytic enzymes such as elastase might damage endothelial cell membranes and the basal lamina, alter the blood-brain barrier, and contribute to the formation of post ischemic edema. In addition, loss of integrity of the endothelial cell basal lamina lining might facilitate the escape of red blood cells and the hemorrhagic transformation of a brain infarct (7,42). The view that leukocytes may cause additional damage to potentially viable tissue during acute cerebral ischemia is indirectly supported by data showing a smaller infarct volume in neutropenic animals than in normal controls (7,43,44).

Serum concentrations of soluble isoforms of adhesion molecules can be demonstrated rapidly shed

from surfaces of the cells (27,45,46). The levels of soluble adhesion molecules after cerebral ischemia vary between individuals, but there was no clear-cut correlation of soluble adhesion molecules levels with clinical findings and infarct size (23).

In addition, ICAM family and selectines have a key role in immun and inflammatory responses especially in the development of the atherosclerotic plaque. In the earliest stage, adhesion molecules attach to leukocytes which migrate into subendothelial space (47). Membran bound cell adhesion molecule-1 (VCAM-1), intercelluler adhesion molecule 1 (ICAM-1) endothelial adhesion molecule (ELAM-1) and E Selectine are expressed endothel, smooth muscle and macrophage cells in atherosclerotic process (47). An earlier change in endothelium is adhesion of leukocytes due to increased expression of ICAM-1 and VCAM-1 (48). These changes induce migration and adhesion of monocyte and formation of foam cells.

In animal studies, increased adhesion molecules were found in atherosclerotic lesions also in diabetic humans (49). Increased studies, it was found that increased adhesion molecule levels are a risk factor acute coroner event and indicator of poor prognosis (50,51).

Therapeutic Approach

Acute leukocyte microvascular occlusion and leukocyte infiltrations into ischemic tissue potentiate ischemic cell damage (15). Animal experiments investigating the effect of anti-adhesion molecule strategies in focal cerebral and spinal cord ischemia showed a beneficial effect in models in which transient focal ischemia was followed by reperfusion, but not in models of permanent ischemia (8,52-58). In addition anti-inflammatory agents could have reduced expression and shedding of adhesion molecules as a result of its anti-inflammatory properties (27). There is not a protective effect of the anti ICAM-1 antibody on ischemic cell damage when used in a model of permanent occlusion. Permanent ischemia as a severe insult may simply overwhelm and nullify any beneficial effect derived from the anti-adhesion molecule therapy (15,58).

Reduction of ischemic injury in the central nervous system after administration of anti ICAM- 1 antibody has been attributed to improved blood flow resulting from reduction of leukocyte endothelial adhesion (15,25). The time course of neutrophil influx into ischemic tissue after middle cerebral artery occlusion in the rat is also different for transient and permanent occlusion (15,59). Leukocytes migration into ischemic area in transient ischemia results earlier (by 1 day) than that in permanent ischemia. Thus after permanent ischemia the inflammatory response may be delayed beyond the time at which it can evoke ischemic cell damage (15,59). The difference in therapeutic efficacy between transient and permanent ischemia may provide a clue to the mechanisms of leukocyte-mediated injury. One proposed mechanisms of leukocyte potentiation of ischemia is microvascular occlusion caused by direct mechanical obstruction and the cytotoxic effects of leukocytes on the endothelium. When leukocytes activated during ischemia the upregulation of both leukocytes and endothelial adhesion molecules and then microvascular obstruction occurs (15). Areas of parenchyma that might be viable when blood flow returns are not adequately reperfused ultimately die. The treatment with anti ICAM-1 antibody may reduce injury by improving blood flow during reperfusion (15,25). In experiments with knockout animals, absence of adhesion molecules reduced infarct volume after transient focal cerebral ischemia (8,60-64).

Treatment with a murine anti-ICAM-1 antibody (enlimomab) has been investigated in patients with acute ischemic stroke but unfortunately the case fatality rate in this trial has been significantly higher in the enlimomab patient group than placebo group (8,65). Addition of enlimomab to whole blood of human volunteers unexpectedly caused activation of neutrophil granulocytes (8,66). Adverse effects of enlimomab may have been caused by immunologic factors, such as a spesific humoral or cytotoxic immune response against the medication because of its murine origin or nonspesific complement activation induced by presentation of Fc fragments (8,67). Therefore, it seems rational not to abandon anti adhesion therapy but to develop anti adhesion strategies that do not activate or interfere with the immun system. We need to prevent or at least minimize the immunogenicity of anti adhesion antibodies, for example, by using f(ab)2 fragments or humanized antibodies (8,68). These positive reults of anti adhesion therapy after reperfusion but not after permanent occlusion, together with the recently proven effectiveness of thrombolytic agents in patients with acute ischemic stroke, have resulted in animal experiments testing

combined administration of tissue plasminogen activator (tPA) and anti adhesion molecule antibodies (8,69-71). The combination of tPA and anti CD18 significantly improved neurological deficits and reduced infarct volume (8,69). Experimental studies of anti adhesion blocking agents have shown that this treatment will work only if there is early reperfusion. Ideally, anti adhesion therapy would be started in the hyperacute stage even before the administration of thrombolytic agents. Therefore the combination of anti adhesion strategies and thrombolytic therapy deserves further investigation (8,72,73).

REFERENCES

1. *Yang Y. Wnts and Wing: Wnt Signaling in vertebrate limb development and musculoskeletal morphogenesis. Birth Defects Research (Part C) 2003; 69: 305-17.*
2. *Taira E, Tsukamoto Y, Kohama K, Maeda M, Kiyama H and Miki N. Expression and involvement of gicerin, a cell adhesion molecule, in the development of chick optic tectum. Journal of Neurochemistry 2004; 88: 891-9.*
3. *Atabekoglu CS, Engin Y, Ustun Y, Aytaç A. Üreme fizyolojisi ve adhezyon molekulleri. Ankara Üniversitesi Tıp Fakultesi Mecmuası 2002; 56: 85-92.*
4. *Springer TA. Adhesion receptors of the immune system. Nature 1990; 34: 425-34.*
5. *Nowinski D, Lysheden AS, Gardner H, Rubin WK, Gerdin B, Ivarsson M. Analysis of gene expression in fibroblasts in response to keratinocyte-derived factors in vitro: Potential implications for the wound healing process. J Invest Dermatol 2004; 122: 216-21.*
6. *Hirohashi S, Kanai Y. Cell adhesion system and human cancer morphogenesis. Cancer Sci 2003; 94: 575-81.*
7. *7. Pantoni L, Sarti C, Inzitari D. Cytokines and cell adhesion molecules in cerebral ischemia experimental bases and therapeutic perspectives. Arterioscler Thromb Vasc Biol 1998;18:503-513.*
8. *Frijns CJM., Kappelle LJ. Inflammatory Cell Adhesion Molecules in Ischemic Cerebrovascular Disease. Stroke 2002; 33: 2115-22.*
9. *Somers WS, Tang J, Shaw GD, Camphausen RT. Insights into the molecular basis of leukocyte tethering and rolling revealed by structures of P- and E-selectin bound to SLe(X) and PSGL-1. Cell 2000; 103: 467-79.*
10. *Ohmori K, Kanda K, Mitsuoka C, et al. E-selectins recognize sialyl 6-sulfo Lewis X, the recently identified L-selectin ligand. Biochem Biophys Res Commun 2000; 278: 90-6.*
11. *Hailiang GE, Ye WEN, Yang G, Betz AL. Increased expression of intercellular adhesion molecule-1 in mouse focal cerebral ischemia model. Chin Med J 2000; 113: 75-9.*
12. *Clark WM, Zivin JA. Antileukocyte adhesion therapy: Preclinical trials and combination therapy. Neurology 1997; 49 (Suppl 4): 32-8.*
13. *Wagner S, Tagaya M, Koziol JA, Quaranta V, del Zoppo GJ. Rapid disruption of an astrocyte interaction with the extracellular matrix mediated by integrin a6b4 during focal cerebral ischemia/reperfusion. Stroke 1997; 28: 858-65.*
14. *Sugimoto K, Honda S, Yamamoto T, et al. Molecular cloning and characterization of a newly identified member of the cadherin family, PB-cadherin. J Biol Chem 1996; 271: 11548-56.*
15. *Zhang RL, Chopp M, Jiang N, et al. Anti-Intercellular Adhesion Molecule-1 Antibody Reduces Ischemic Cell Damage After Transient But Not Permanent Middle Cerebral Artery Occlusion in the Wistar Rat. Stroke 1995; 26: 1438-43.*
16. *Barone FC, Schmidt DB, Hillegass LM, et al. Reperfusion increases neutrophils and leukotriene B4 receptor binding in rat focal ischemia. Stroke 1992; 23: 1337-48.*
17. *Chen H, Chopp M, Zhang ZG, Garcia JH. The effect of hypothermia on the transient cerebral artery occlusion in the rat. J Cereb Blood Flow Metab 1992; 12: 621-8.*
18. *Kochanek PM, Hallenbeck JM. Polymorphonuclear leukocytes and monocytes/macrophages in the pathogenesis of cerebral ischemia and stroke. Stroke 1992; 23: 1367-79.*
19. *Jean WC, Spellman SR, Nussbaum ES, Low WC. Reperfusion injury after focal cerebral ischemia: The role of inflammation and the therapeutic horizon. Neurosurgery 1998; 43: 1382-96.*
20. *Clark RK, Lee EV, Fish CJ, et al. Development of tissue damage, inflammation and resolution following stroke: An immunohistochemical and quantitative planimetric study. Brain Res Bull 1993; 31: 565-72.*
21. *21. Clark RK, Lee EV, White RF, Jonak ZL, Feuerstein GZ, Barone FC. Reperfusion following focal stroke hastens inflammation and resolution of ischemic injury. Brain Res Bull 1994;35:387-392.*
22. *Zhang RL, Chopp M, Chen H, Garcia JH. Temporal profile of ischemic tissue damage, neutrophil response, and vascular plugging following permanent and transient (2H) middle cerebral artery occlusion in the rat. J Neurol Sci 1994; 125: 3-10.*
23. *Bitsch A, Klene W, Murtada L, prange H, Rieckmann P. A longitudinal prospective study of soluble adhesion molecules in acute stroke. Stroke 1998; 29: 2129-35.*
24. *Garcia JH, Liu KF, Yoshida Y, Lian J, Chen S, del Zoppo GJ. Influx of leukocytes and platelets in an evolving brain infarct (Wistar rat). Am J Pathol 1994; 144: 188-99.*
25. *Clark WM, Madden KP, Rothlein R, Zivin JA. Reduction of central nervous system ischemic injury by monoclonal antibody to intercellular adhesion molecule. J Neurosurg 1991; 75: 623-7.*
26. *Frenette PS, Wagner DD. Adhesion molecules, part I. N Engl J Med 1996; 334: 1526-9.*

27. Fassbender K, Bertsch T, Mielke O, Muhlhauser F, Hennerici M. Adhesion molecules in cerebrovascular diseases evidence for an inflammatory endothelial activation in cerebral largeand small-vessel disease. Stroke 1999; 30: 1647-50.

28. Rothlein R. Overview of leukocyte adhesion. Neurology 1997; 49 (Suppl 4): 3-4.

29. Howard MC, Miyajima A, Coffmann R. T-cell-derived cytokines and their receptors. In: We P, ed. Fundamental Immunology. 3rd ed. New York, NY: Raven Press Ltd; 1993: 763-800.

30. Lechan RM, Clark TBD, Cannon JG, Shaw AR, Dinarello CA, Reichlin S. Immunoreactive IL-1b localization in the rat forebrain. Brain Res 1990; 514: 135-40.

31. Conway EM, Bach R, Rosenberg RD, Konigsberg WH. Tumor necrosis factor enhances expression of tissue factor mRNA in endothelial cells. Thromb Res 1989; 53: 231-41.

32. Chappel DC, Varner SE, Nerem RM, Medford RM, Alexander RW. Oscillatory shear stress stimulates adhesion molecule expression in cultured human endothelium. Circ Res 1998; 82: 532-9.

33. Allen S, Khan S, Al-Mohanna F, Batten P, Yacoub M. Native low density lipoprotein-induced calcium transients trigger VCAM-1 and E-selectin expression in cultured human vascular endothelial cells. J Clin Invest 1998; 101: 1064-75.

34. Kuo CC, Grayston JT, Campbell LA, Goo YA, Wissler RW, Benditt EP. Chlamydia pneumoniae (TWAR) in coronary arteries of young adults (15-34 years old). Proc Natl Acad Sci U S A 1995; 92: 6911-4.

35. Wimmer MLJ, Sandmann-Strupp R, Saikku P, Haberl RL. Association of chlamydial infection with cerebrovascular disease. Stroke 1996; 27: 2207-10.

36. Alexander RW. Inflammation and coronary artery disease. N Engl J Med 1994; 331: 468-9.

37. del Zoppo GJ, Schmidt-Schonbein GW, Mori E, Copeland BR, Chang CM. Polymorphonuclear leukocytes occlude capillaries following middle cerebral artery occlusion and reperfusion in baboons. Stroke 1991; 22: 1276-83.

38. Cengiz N, Colakoglu N, Kavakli A, Sahna E, Parlakpinar H, Acet A. Effects of caffeic acid phenethyl ester on cerebral cortex: Structural changes resulting from middle cerebral artery ischemia reperfusion. Clinical Neuropathology 2007; 26: 80-4.

39. Sutton DW, Schmid-Schonbein GW. Elevation of organ resistance due to leukocyte perfusion. Am J Physiol 1992; 262: H1648-50.

40. Hartl R, Schurer L, Schmid-Schonbein GW, del Zoppo G. Experimental antileukocyte interventions in cerebral ischemia. J Cereb Blood Flow Metab 1996; 16: 1108-19.

41. Hamann GF, del Zoppo GJ. Leukocyte involvement in vasomotor reactivity of the cerebral vasculature. Stroke 1994; 25: 2117-9.

42. Hamann GF, Okada Y, del Zoppo GJ. Hemorrhagic transformation and microvascular integrity during focal cerebral ischemia/reperfusion. J Cereb Blood Flow Metab 1996; 16: 1373-8.

43. Matsuo Y, Onodera H, Shiga Y, Nakamura M, Ninomiya M, Kihara T, Kogure K. Correlation between myeloperoxidasequantified neutrophil accumulation and ischemic brain injury in the rat: Effects of neutrophil depletion. Stroke 1994; 25: 1469-75.

44. Connolly ES Jr, Winfree CJ, Springer TA, et al. Cerebral protection in homozygous null ICAM-1 mice after middle cerebral artery occlusion: role of neutrophil adhesion in the pathogenesis of stroke. J Clin Invest 1996; 97: 209-16.

45. Carlos TM, Harlan JM. Leukocyte-endothelial adhesion molecules. Blood 1994; 84: 2068-101.

46. Gearing AJH, Newman W. Circulating adhesion molecules in disease. Immunology Today 1993; 14: 506-12.

47. Munro JM, Cotran RS. Biology of disease: The pathogenesis of atherosclerosis: Atherogenesis and inflammation. Lab¯Invest 1988; 58: 249-61.

48. O'Brien KD, McDonald TO, Chait A, et al. Neovasculer expression of E-Selectin, intercelluler adhesion molecule-1, and vasculer cell adhesion molecule-1 in human atherosclerosis and their relation to intimal leukocyte content. Circulation 1996; 93: 672-82.

49. Inhibashi S, Goldstain JL, Brown MS, et al. Massive xanthomatosis and atherosclerosis in cholesterol-fed low density lipoprotein receptor-negative mice. J Clin Inwest 1994; 93: 1885-93.

50. Pellegatta F, Pizzetti G, Lu Y, et al. Soluble E-Selectin and intracelluler Molecule-1 plasma levels increase during acute myocardial infarction. J Cardiovasc Pharmacol 1997; 30: 455-60.

51. Ridker PM, Hennekens CH, Roitman-Johnson B, et al. Plasma consentration of soluble intercelluler adhesion molecule 1 and risks of future myocardial infarction in apperantly healthy men. Lancet 1998; 351: 88-92.

52. Huang J, Choudhri TF, Winfree CJ, et al. Postischemic cerebrovascular E-selectin expression mediates tissue injury in murine stroke. Stroke 2000; 31: 3047-53.

53. 53. Chopp M, Li Y, Jiang N, Zhang RL, Prostak J. Antibodies against adhesion molecules reduce apoptosis after transient middle cerebral artery occlusion in rat brain. J Cereb Blood Flow Metab 1996;16:578-584.

54. Morikawa E, Zhang SM, Seko Y, Toyoda T, Kirino T. Treatment of focal cerebral ischemia with synthetic oligopeptide corresponding to lectin domain of selectin. Stroke 1996; 27: 951-5.

55. Goussev AV, Zhang Z, Anderson DC, Chopp M. P-selectin antibody reduces hemorrhage and infarct volume resulting from MCA occlusion in the rat. J Neurol Sci 1998; 161: 16-22.

56. Relton JK, Sloan KE, Frew EM, Whalley ET, Adams SP, Lobb RR. Inhibition of alpha4 integrin protects against transient focal cerebral ischemia in normotensive and hypertensive rats. Stroke 2001; 32: 199-205.

57. Becker K, Kindrick D, Relton J, Harlan J, Winn R. Antibody to the 4 integrin decreases infarct size in transient focal cerebral ischemia in rats. Stroke 2001; 32: 206-11.

58. Ferrante A, Kowanko IC, Bates EJ. Mechanisms of host tissue damage by cytokine-activated neutrophils. Immunol Ser 1992; 57: 499-521.

59. Zhang RL, Chopp M, Chen H, Garcia JH. Temporal profile of ischemic tissue damage, neutrophil response, and vascular plugging following permanent and transient (2H) middle cerebral artery occlusion in the rat. J Neurol Sci 1994; 125: 3-10.

60. Prestigiacomo CJ, Kim SC, Connolly ES Jr, Liao H, Yan SF, Pinsky DJ. CD18-mediated neutrophil recruitment contributes to the pathogenesis of reperfused but not nonreperfused stroke. Stroke 1999; 30: 1110-7.

61. Soriano SG, Coxon A, Wang YF, et al. Mice deficient in Mac-1 (CD11b/CD18) are less susceptible to cerebral ischemia/reperfusion injury. Stroke 1999; 30: 134-9.

62. Kitagawa K, Matsumoto M, Mabuchi T, et al. Deficiency of intercellular adhesion molecule-1 attenuates microcirculatory disturbance and infarction size in focal cerebral ischemia. J Cereb Blood Flow Metab 1998; 18: 1336-45.

63. 63. Connolly ES Jr, Winfree CJ, Springer TA et al. Cerebral protection in homozygous null ICAM-1 mice after middle cerebral artery occlusion: role of neutrophil adhesion in the pathogenesis of stroke. J Clin Invest 1996; 97: 209-6.

64. Connolly ES Jr, Winfree CJ, Prestigiacomo CJ et al. Exacerbation of cerebral injury in mice that express the Pselectin gene: Identification of P-selectin blockade as a new target for the treatment of stroke. Circ Res 1997; 81: 304-10.

65. Enlimomab Acute Stroke Trial. Use of anti-ICAM-1 therapy in ischemic stroke: Results of the Enlimomab Acute Stroke Trial. Neurology 2001; 57: 1428-34.

66. Vuorte J, Lindsberg PJ, Kaste M, et al. Anti-ICAM-1 monoclonal antibody R6.5 (enlimomab) promotes activation of neutrophils in whole blood. J Immunol 1999; 162: 2353-7.

67. DeGraba TJ. The role of inflammation after acute stroke: Utility of pursuing anti-adhesion molecule therapy. Neurology 1998; 51 (Suppl 3): 62-8.

68. DeGraba TJ, Pettigrew LC. Why do neuroprotective drugs work in animals but not humans? Neurol Clin 2000; 18: 475-93.

69. Zhang RL, Zhang ZG, Chopp M, Zivin JA. Thrombolysis with tissue plasminogen activator alters adhesion molecule expression in the ischemic rat brain. Stroke 1999; 30: 624-9.

70. Zhang RL, Zhang ZG, Chopp M. Increased therapeutic efficacy with rt-PA and anti-CD18 antibody treatment of stroke in the rat. Neurology 1999; 52: 273-9.

71. Bednar MM, Gross CE, Russell SR, et al. Humanized anti-Lselectin monoclonal antibody DREG200 therapy in acute thromboembolic stroke. Neurol Res 1998; 20: 403-8.

72. Zivin JA. Thrombolytic stroke therapy: Past, present, and future. Neurology 1999; 53: 14-9.

73. Lindsberg PJ, Roine RO, Tatlisumak T, Sairanen T, Kaste M. The future of stroke treatment. Neurol Clin 2000; 18: 495-510.