Adhesion Molecules in Cerebral Ischemia and Atherosclerosis

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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.

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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 immunoglobulin 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 α- and ß-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 α 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 lig gene superfamily molecules (8,9,10,12).

Leukocytes and monocytes also express the integrin α4β1 (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 interaction between astrocytes and extracellular matrix, is rapidly damaged during focal cerebral ischemia/reperfusion. Other integrins 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 specificity 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, α-, β-, 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 polymorphonuclear 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 leukocytes 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 mediators of immunologic and inflammatory responses that may have various functions during cerebral ischemia: on the one hand, they can attract leukocytes 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 plasminogen activating 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 transeendothelial migration mediated by ICAM-1 or Eselectin may be responsible for vascular injury by release of reactive oxygen metabolites, granular enzymes 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 adherence 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 coro- ner 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 specifc humoral or cytotoxic immune response against the medication because of its murine origin or nonspecific 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 adhesive 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 adhesive blocking agents have shown that this treatment will work only if there is early reperfusion. Ideally, anti adhesive therapy would be started in the hyperacute stage even before the administration of thrombolytic agents. Therefore the combination of anti adhesive strategies and thrombolytic therapy deserves further investigation (8,72,73).

REFERENCES


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