CELL ADHESION MOLECULES AND ISCHEMIC STROKE

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Abstract

Inflammation has been implicated in the pathogenesis of ischemic stroke and the recruitment of inflammatory cells appears to exacerbate ischemic brain injury. Since leukocyte-endothelial cell adhesion is a rate-determining step in the recruitment of leukocytes into post-ischemic brain tissue, much attention has been devoted to defining the contribution of different adhesion molecules, expressed either on leukocytes or endothelial cells, to the leukocyte recruitment process. Similarly, a large effort has been made to determine whether interference with leukocyte-endothelial cell adhesion protects the brain against ischemic tissue injury. These efforts have revealed an important contribution of $\beta2$-integrins (CD11/CD18), ICAM-1, and P-selectin in the recruitment of leukocytes as well as platelets in the post-ischemic cerebral microvasculature. Immunoblockade or genetic deletion of these adhesion molecules has been shown to reduce infarct volume, edema, behavioral deficits, and/or mortality in different animal models of ischemic stroke. Anti-adhesion agents also appear to widen the therapeutic window for thrombolytic therapy in these experimental models. Emerging evidence on the role of signaling pathways (eg, CD40/CD40L, Notch-1) and immune cells in the regulation of ischemia-reperfusion induced leukocyte recruitment in the cerebral microvasculature offer novel targets for controlling inflammation in stroke. The few clinical trials assessing anti-adhesion therapy in ischemic stroke have all failed to show efficacy. It remains to be determined whether inflammation in general and leukocyte adhesion in particular represent useful targets for therapeutic intervention in stroke patients.

Introduction

Stroke is defined as "rapidly developing clinical signs of focal or global disturbance of cerebral function with symptoms lasting 24 hours or longer, or leading to death with no apparent cause other than of vascular originrdquo; (1) Although this definition includes the hemorrhagic forms of stroke, 80 % of stroke cases occur due to the occlusion of arteries carrying blood to the brain and subsequent ischemia. Ischemic stroke is the third leading cause of death in the United States with an estimated cost of 71.8 billion dollars (2). The mortality rate after an ischemic incident is very high 30% and survivors almost always face disabilities that require costly long term care (3).

Despite the high mortality and morbidity associated with ischemic stroke, current established therapies are limited. To date, the only effective treatment approved for acute ischemic stroke in the U.S. and Canada is thrombolysis achieved by recombinant tissue plasminogen activators

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(rt-PA). However, this regime needs to be applied within 3 hour of symptom onset, decreasing the availability of treatment to the majority of patients in need (4). In addition to thrombolysis, anti-platelet therapies such as aspirin and glycoprotein IIb-IIIa inhibitors (clopidogrel) or anticoagulants (heparin) have been used in the prevention/treatment of acute ischemic stroke. Aspirin treatment is associated with significantly fewer recurrent ischemic strokes and no significant increase in hemorrhagic strokes at 14 days. A small but a significant improvement at 6 months has also been observed with aspirin in large-scale clinical studies. Heparin treatment, however, does appear to offer any clinical advantage at 6 months (5), and initial efforts to assess glycoprotein IIb/IIIa directed treatment strategies have not shown promising results (6).

After an ischemic insult, the neuronal injury around the ischemic core, called the penumbra, continues to develop over several hours. Neuronal tissue within the penumbra is electrically inactive but viable, and considered to represent salvageable tissue that can be targeted with neuroprotective interventions. The slow evolution of ischemic damage within the penumbra provides a window of opportunity for neuroprotective therapies. Attenuating and/or delaying this time-dependent brain injury may improve neurological outcome and facilitate brain recovery from injury (7).

Experimental interventions that have been used to confer protection to the penumbra include free radical scavengers and synthesis inhibitors, excitotoxicity inhibitors, suppressors of neuronal metabolism (e.g. hypothermia), anti-inflammatory agents, and membrane stabilizers (8). While there is substantial experimental evidence demonstrating the beneficial effects of these interventions in animal models, human trials have either failed or proven inadequate (9,10).

Anti-Inflammation as a Therapeutic Target for Ischemic Stroke

Ischemic stroke frequently results from thromboemboli blocking the blood supply to neuronal tissue. Immediately after cessation of blood flow, due to the high oxygen and nutrient needs of brain tissue, ATP depletion occurs in the neurons. Consequently, the ionic gradients across the cellular membranes cannot be sustained resulting in calcium and water influx and neurotransmitter release. This sequence of events leads to cytotoxic edema, excitotoxicity and activation of intracellular enzymes. The overall impact of blood flow cessation is cellular damage and initiation of an inflammatory response. While the other triggering events for cellular damage occurs rapidly after the stroke, inflammation occurs over hours to days and provides an excellent window of opportunity for new treatment strategies (11).

Many studies demonstrate that cerebral ischemia is associated with the infiltration of inflammatory cells to the ischemic region (12–20). Infiltration of the ischemic brain region by leukocytes is associated with inflammatory activation of cerebral endothelial cells, microglia/ macrophages and astrocytes (3). Activation of these resident cell populations along with immune cells stimulates the production and release of pro-inflammatory cytokines such as TNF-α and IL-1 from the ischemic tissue (22). In this inflammatory environment, cerebral endothelial cells increase their expression of cell surface adhesion molecules that mediate recruitment of leukocytes and platelets to the ischemic region (22–26). A role for leukocytes in the pathogenesis of post-ischemic brain injury is supported by three major lines of evidence: 1) leukocytes (neutrophils and/or lymphocytes) accumulate in the brain prior to or during the onset of tissue injury, 2) depletion of neutrophils from the circulation reduces infarct volume and improves neurological outcome, and 3) immunoneutralization or genetic deletion of cell adhesion molecules that mediate leukocyte recruitment reduces tissue injury and brain dysfunction in animal models of focal and global cerebral ischemia (27–38).

Brain ischemia triggers profound changes in cerebral microvascular endothelium. In microvessels, the endothelial cell activation is accompanied by an upregulation of adhesion molecules, which promotes the rolling, and firm adhesion of both leukocytes and platelets. The leukocyte recruitment may lead to further injury by releasing reactive oxygen species, proteases and inflammatory mediators. The adhesion process may also trigger signaling cascades in cerebral endothelial cells, leukocytes or both which may contribute to the injury (39). The accumulation of platelets, either directly attached to endothelial cells or bound to adherent leukocytes promote a prothrombotic state that further exaggerates the perfusion deficit and state of "no-reflow" after cerebral ischemia (32).

Although there are a large number of reports that implicate leukocytes in the tissue injury associated with ischemic stroke, there are also several reports that do not support this contention (40,41,42,43). For example, it has been reported that brain necrosis precedes the appearance of neutrophils and that further increases in the number of neutrophils in the infarcted region is not associated with a larger infarct size (40). Furthermore, some studies have revealed an inability of neutrophil blocking antibodies to protect against neuronal tissue injury following ischemia reperfusion (I/R) (42,43). These observations lead to the conclusion that neutrophils may be by-standers rather that the effectors of the tissue injury induced by cerebral ischemia (15). This view is also supported by studies that fail to demonstrate protection against ischemic brain injury in adhesion molecule deficient mice (44,45). These inconsistent findings may be explained by the differences in experimental models/techniques or by the duration and magnitude of the ischemic insult and reperfusion periods.

Leukocyte Adhesion in Postischemic Cerebral Microvessels

The recruitment of leukocytes and platelets in the cerebral microvasculature is widely regarded as a rate-limiting step in the inflammatory response associated with cerebral ischemia. Under normal conditions (non-inflamed) cerebral endothelium is a poor substrate for the rolling and firm adhesion of circulating cells. Intravital microscopy studies have revealed that the number of rolling leukocytes in normal (non-inflamed) cerebral venules is very low compared to skeletal muscle and mesenteric (46). However, in response to an ischemic (or other inflammatory) insult cerebral endothelium are capable to expressing high levels of adhesion molecules and recruit large numbers of leukocytes.

The recruitment of leukocytes and platelets in cerebral venules exposed to ischemia and reperfusion is a highly coordinated and well-regulated process that involves different adhesion molecules expressed on vascular endothelium and circulating cells (Figure 1). This recruitment process involves at least two different stages, i.e., an initial low affinity binding that is manifested as rolling and a later high affinity interaction that results in firm adhesion. The rolling interaction appears to be largely mediated by P-selectin expressed on endothelial cells that engage with sialyl Le^x moieties on circulating cells (47,48), while firm adhesion is mediated by an interaction between β2-integrins on leukocytes with ICAM-1 (49) on cerebral microvascular endothelial cells. Both CD11a/CD18 (LFA-1) or CD11b/CD18 (Mac-1) seem to play a role in the firm adhesion of leukocytes at 24 h of reperfusion of ischemia since CD11a (LFA-1) or CD11b (Mac-1) knock out mice exhibit less accumulation of adherent leukocytes in cerebral venules at 24 hrs (but not at 4 hrs) of reperfusion following occlusion of the middle cerebral artery (50).

Leukocyte adhesion has been demonstrated in different experimental models of cerebral ischemia and hypoxia (51,52,53,54). In these experiments leukocyte adhesion has been observed as early as 30 min after reperfusion and as late as 48 hours (54,55). These kinetics are consistent with a report describing P-selectin upregulation as early as 15 min following an ischemic insult, with increased E-selectin expression occurring within 2 h of ischemia (56).

The intensity of the leukocyte adhesion response appears to depend on the nature of the ischemic/hypoxic insult. For example, asphyxia elicits less leukocyte adhesion (57) compared to focal and global ischemia (54,47) in brain microvessels. In the early stages of reperfusion after ischemia, neutrophils represent the dominant leukocyte population that accumulates at the cerebral microvasculature. This view is supported by studies showing that rendering mice neutropenic anti-neutrophil serum attenuates the recruitment of leukocytes in cerebral venules at 4 hours after I/R but has no effect on leukocyte adhesion at 24 hrs after reperfusion (54). It appears likely that the leukocyte population that accumulates at 24 hrs of reperfusion is mainly mononuclear cells rather than neutrophils (54,58).

Platelet Adhesion in Postischemic Cerebral Microvessels

Increased leukocyte adhesion after cerebral I/R is also accompanied by the recruitment of rolling and adherent platelets. Immunoblockade of the platelet adhesion molecule glycoprotein/IIb/IIIa has no effect on the platelet adhesion elicited by cerebral I/R. However, adhesion molecule blocking antibodies that inhibit leukocyte adhesion, such as P-selectin, CD18, and ICAM-1, in postischemic cerebral venules also blunt the recruitment of adherent platelets (47,59). Mice deficient in either endothelial cell or platelet P-selectin exhibit a blunted platelet recruitment response to I/R (54,47). Collectively, the data generated using different blocking antibodies and mutant mice are consistent with a model of platelet recruitment that involves the binding of platelet-associated P-selectin to its ligand PSGL-1 on leukocytes, which in turn utilize endothelial cell P-selectin and ICAM-1 to attach to the venular walls. This dependency of platelet adhesion on leukocyte adhesion is consistent with the observation that platelet accumulation lags behind the leukocyte accumulation (60) in postischemic cerebral venules and that a large proportion of platelets bind to already adherent leukocytes (61). While the consequences of platelet accumulation to the overall injury response remain poorly understood, there is evidence suggesting the binding of platelets to adherent leukocytes may lead to further activation of both cell types, which may exacerbate the injury response (54,62). The results of a recent clinical study of genetic variants of PSGL-1 and P-selectin genes have revealed an association between some variants and the risk for ischemic stroke, however it remains unclear if and how this genetic association relates to platelet-leukocyte-endothelium interactions (63).

T-Lymphocytes, Blood Cell Adhesion and Stroke

There is a growing body of evidence in the literature that supports a role for T-lymphocytes in the tissue injury following an ischemic stroke. (58,64,65) In a mouse model of cerebral ischemia-reperfusion, T-lymphocytes appear to be major modulators of the adhesion of leukocytes and platelets in cerebral venules, and infarct size. Mice deficient in either CD4+ and/or CD8+ T-lymphocytes exhibit a reduced infarct size, lower number of adherent leukocytes and platelets in the cerebral venules, and improved neurological outcome at 24 hours after reperfusion (58). B-lymphocyte deficient mice did not exhibit protection against the inflammation, tissue injury, and neurological deficit induced by ischemic stroke. While these observations implicate T (but not B-)-lymphocytes in the pathogenesis of experimental stroke, there are no in vivo studies to date that directly demonstrate the adhesion of T-lymphocytes to cerebral endothelium after ischemia. However, in vitro experiments have revealed that the adhesion of lymphocytes, derived from stroke patients, to cerebral endothelium is significantly higher than that noted for lymphocytes isolated from healthy subjects (94). The pathophysiological relevance of this observation is unclear since it is not known whether T-lymphocytes must directly interact with cerebral endothelial cells to exert their deleterious actions during ischemic stroke.

Modulation of Blood Cell Adhesion

Several agents and interventions have been shown to alter the adhesion of platelets and leukocytes in cerebral vessels following I/R. These include physical factors, pharmacological agents, interventions targeting the blockade of adhesion molecules and some of the risk factors for cardiovascular diseases. High venular shear rates (18), platelet activating factor receptor antagonists (51), hypothermia (52), hydroxyethyl starch (57), and estrogen (66) decreases the leukocyte accumulation, while low venular shear rates (61) and ovariectomy increases the leukocyte adhesion into cerebral vessels following I/R.

Some of the risk factors for cardiovascular disease also appear to modulate the adhesion of platelets and leukocytes in the cerebral vessels after I/R. For example, hypercholesterolemia increases the number of adherent leukocytes and platelets in cerebral venules after I/R (59). Evidence from postischemic retinal microvessels suggests that hypertension may also exacerbate the adhesion of leukocytes and platelets following I/R in the CNS (67). A recent study has addressed the contribution of angiotensin II (Ang II) type 1 receptor signaling to platelet-leukocyte-endothelial cell interactions in the cerebral microvasculature. ANG II type 1 signaling results in increased adhesion of leukocytes and platelets by a P-selectin and PSGL-1 mediated mechanism and may represent a mechanism that links hypertension to tissue injury in ischemic stroke (68).

Therapeutic targeting of leukocyte-endothelial cell adhesion

In several experimental studies, adhesion molecules were targeted in order to define the mechanisms of cell recruitment and the related tissue damage after cerebral I/R (Table 1). These interventions include immunoblockade of adhesion molecules with specific antibodies, inhibition with ligands or antagonists, and genetic deletion. The principal targets for these interventions include the selectins, ICAM-1 and the β2-integrins, LFA-1 and Mac-1.

Selectin blockade

Following cerebral ischemia, P- and E-selectins are highly expressed at brain. P-selectin can be detected as early as 15 min after reperfusion while E-selectin expression is observed beginning at 2 h after ischemia. The expression of selectins contributes to the early recruitment of circulating cells to the infarct region (56). The link between P-selectin expression and injury following ischemic stroke seems to involve a complement-dependent pathway wherein P-selectin upregulation results from complement activation and can be modulated with a targeted approach to complement receptor 2 without an effect on systemic complement activity (69).

The polysaccharide fucoidin (FCN), a homopolymer of sulfated L-fucose that competitively inhibits P- and L-selectin, has been shown to attenuate the leukocyte accumulation during reperfusion of the rat brain following focal ischemia. Fucoidin also reduced infarct size and improved neurological outcome, suggesting a role for selectin-dependent leukocyte-endothelial cell interactions following cerebral ischemia (70). However, L-selectin blockade with a humanized anti-L-selectin antibody did not decrease the tissue damage or number or infiltrating leukocytes to the ischemic region in a rabbit model of transient cerebral ischemia (71). In another study employing a similar cerebral I/R model, anti-L-Selectin antibodies were found to be effective only when used in combination with tissue plasminogen activators (tPA), which addresses the potential involvement of L-selectin in tissue injury following thrombolytic reperfusion of ischemic brain (72).

Therapies targeting P-selectin have also been shown to be effective in different experimental settings. In a model of cerebral I/R, P-selectin knockout mice exhibited a reduction in infarct volume, better functional outcome and a better return of cerebral blood flow after ischemia

(36). Similarly blocking antibodies for P-selectin reduced infarct size with a reduced hemorrhagic transition in cerebral I/R (73). In a permanent ischemia model, P-selectin immunoblockade attenuated both infarct size and brain edema, which were associated with a reduction of leukocyte infiltration (35). In these studies, the anti-P-selectin antibodies were administered 30 min before the ischemic insult, which lessens the therapeutic value of the observed protection.

E-selectin immunoblockade does not need to be done during the pre-ischemic period, thereby enhancing it's utility as a potential therapeutic strategy. Blocking E-selectin with specific antibodies as long as 90 minutes after onset of ischemia has been shown to reduce infarct size (74). In a rat model of transient ischemia, E-selectin blockade with an analogue of sialyl-LewisX (CY-1503) reduced both infarct size and neutrophil infiltration. The possibility that CY-1503 affords protection by blocking L-selectin on leukocytes or P-selectin on endothelial cells or platelets can not be discounted (30).

A blinded placebo-controlled trial in a nonhuman primate model of reperfused stroke has been performed to test the combination of blocking of both E- and P-selectin using a humanized monoclonal antibody (HuEP5C7). Administration of the blocking antibody during the occlusion period reduced infarct volume and improved neurological outcome. No immune response (complement activation) was noted with the antibody. Despite the promising outcome of this study, there is no evidence supporting the effectiveness HuEP5C7 if it is administered after onset of the stroke (75). Finally, blocking the lectin domain of selectins with a synthetic oligopeptide has been shown to reduce brain injury in a transient, but not in a permanent, model of cerebral ischemia, suggesting that reperfusion is necessary for effective anti-selectin therapy (34).

Induction of mucosal immune tolerance to E-selectin represents another promising strategy for prevention of ischemic and hemorrhagic stroke. Takeda et al. have demonstrated that the induction of mucosal tolerance to E-selectin, by repeated nasal instillation of this antigen, prevents the development of ischemic and hemorrhagic strokes in spontaneously hypertensive stroke-prone rats (76,77). In a follow-up study, the protective effects of mucosal tolerance to E-selectin were also demonstrated in a permanent model of cerebral ischemia. In the latter study, the neuroprotective effects of mucosal tolerance to E-selectin was linked to immunomodulation of CD8+ T-cell responses addressing a possible link between T-cell modulation and neuroprotection. A better understanding of this link may provide promising selective therapeutic options in stroke (78).

ICAM-1 blockade

The results of several studies have revealed significant ICAM-1 expression in the postischemic brain and a role for this adhesion molecule in the recruitment of inflammatory cells (78–82). In vitro experiments confirm an increase in ICAM-1 in cerebral endothelial cells following ischemia or ischemia-like conditions (25,83,84). Human studies provide further support for an increased ICAM-1 expression in the ipsilateral cortex and increased circulating levels of soluble ICAM-1 following ischemic stroke (85,86).

ICAM-1 expression is an essential step in mediating the firm adhesion of leukocytes in cerebral microvessels after ischemic stroke and there are several studies that address the contribution of ICAM-1 to cerebral injury after stroke (38,87,88,89,90). ICAM-1 knockout mice exhibit a reduction in leukocyte adhesion, smaller infarcts, decreased leukocyte adhesion (87), improved cerebral blood flow and lower mortality after cerebral ischemia and reperfusion (38). Similarly, ICAM-1 immunoblockade reduces ischemic brain injury and neutrophil accumulation in both rat (88,90) and rabbit (89) models of cerebral ischemia. The combined use of anti-ICAM antibodies with tPA has been shown to improve neurological outcome under conditions

wherein neither of these interventions are independently effective, indicating that ICAM-1 blockade may improve outcome of tPA administration with an extended therapeutic window for thrombolysis (91).

LFA-1/MAC-1 blockade

LFA-1 (CD11a/CD18) and Mac-1 (CD11b/CD18) are β2-integrins that mediate the firm adhesion of leukocytes on vascular endothelium. LFA-1 is expressed on all leukocytes where Mac-1 expression is found on neutrophils, monocytes and natural killer cells. Both adhesion molecules are constitutively expressed and may exhibit increased expression upon stimulation. ICAM-1 and -2 on endothelial cells are known targets for LFA-1 and Mac-1 (92). The expression of CD11a and CD18 is upregulated in stroke patients and patients with transient ischemic attacks for up to 72 hours after the ischemic incident, revealing an association between cerebral ischemia and the cell surface density of these adhesion molecules (93).

Mac-1 and CD18 knockout mice subjected to stroke followed by reperfusion exhibit a reduced infarct volume and lower mortality, compared to their wild type counterparts (37,45). In models of transient cerebral ischemia, immunoblockade of either CD11b, CD18, or Mac-1 (28,33, 95,96) also affords protection against tissue injury. Similarly, CD18 immunoneutralization in rats reduces the edema, leukocyte infiltration and infarct size resulting from transient ischemia (28). In an in vitro study, the enhanced adhesion of lymphocytes to cerebral endothelial cells appears to be dependent to LFA-1 and ICAM-1 expression following ischemic stroke. Furthermore, lymphocytes isolated from patients with stroke exhibit increased adhesion to cerebral endothelium when compared to lymphocytes derived from healthy donors (94). The contributions of LFA-1 and Mac-1 to I/R-induced brain injury and cerebral microvascular dysfunction at 4 and 24 hrs after reperfusion have been addressed using mice deficient in either leukocyte adhesion molecule. Both mutants exhibited smaller infarcts, improved neurological outcome, and less adhesion of leukocytes and platelets in cerebral microvessels. Interestingly, the lack of LFA-1 or Mac-1 had no effect on leukocyte adhesion at 4 hours of reperfusion but reduced the number of adherent leukocytes at 24 h, suggesting a more important role for these adhesion molecules at later times after reperfusion (50).

Anti-adhesion molecule strategies in ischemic stroke have proven more effective following transient, but not permanent, ischemia (45,97,98). This observation suggests that anti-adhesion interventions may offer more therapeutic benefit in patients receiving rt-PA to achieve reperfusion following ischemic stroke. Support for this possibility is provided by the observation that a combination of anti-β2-integrin (CD11/CD18) and tPA extends the therapeutic window of tPA, with a better outcome than the additive effects of these agents in experimental animals (99,100).

Leukocyte adhesion and signaling pathways

Ischemia elicits changes in different signaling pathways in endothelial cells that allow for the recruitment of leukocytes and platelets in cerebral venules. Increased production of reactive oxygen species (ROS) following I/R is considered to be one of the major mechanisms that links I/R and increased adhesion of blood cells in cerebral microvessels. Enhanced superoxide production is associated with cerebral I/R and this response is exacerbated by hypercholesterolemia (59) and diabetes mellitus (101). NADPH oxidase appears to be a major source of ROS production in the postischemic cerebral microvasculature (59,68). ROS are known to trigger the expression of endothelial cell adhesion molecules that mediate leukocyte adnd platelet adhesion in postischemic cerebral venules. Superoxide may promote adhesion by inactivating nitric oxide, which is an endogenous inhibitor of leukocyte adherence (102). Nitric oxide donors such as nitroprusside inhibit leukocyte adhesion (103). The opposing actions of superoxide and nitric oxide suggest that the balance between these reactive species

in the cerebral microcirculation may be a major determinant of the blood cell adhesion induced by I/R.

CD40-CD40L signaling also appears to play a major role in modulating the recruitment of blood cells in brain microvessels following stroke (53). CD40 and CD40L knockout mice exhibit a smaller infarct volume and lower numbers of rolling and adherent leukocytes and platelets in postischemic venules. While the diminished blood cell recruitment that is observed in CD40-CD40L knockout mice may be a secondary consequence of the smaller infarct volume, a direct effect on blood cell-interactions is also a likely explanation for the protection afforded by these mutations.

Notch-1 is a cell surface receptor that controls a broad number of cellular processes such as cell differentiation, proliferation and apoptosis. The Notch-1 signaling pathway also mediates angiogenesis, T-cell activation and proliferation, synaptic plasticity and cell-fate decisions in nervous system (104,105). A recent study addressed the contribution of Notch-1 signaling to the inflammatory and tissue injury responses to cerebral I/R (106). Mice treated with inhibitors of the Notch activating enzyme, gamma-secretase, as well as Notch antisense transgenic mice exhibit an attenuated brain injury response to I/R. This protective effect was accompanied by a blunted leukocyte/platelet recruitment response and a reduced number of ICAM-1 and CD11b positive cells in ischemic brain.

Clinical Trials Employing Anti-Adhesion Strategies

ICAM-1 blockade (Enlimomab)

The promising data obtained with anti-adhesion molecule strategies in different animal models of ischemic stroke lead to clinical trials that tested the efficacy of anti-ICAM-1 antibodies in human stroke. The murine monoclonal anti-ICAM-1, Enlimomab was administered over 5 days during an observation period of 30 +/− 10 days and initially found to be safe for use in humans with ischemic stroke (107). However, in a prospective, randomized, blinded, phase III trial, administration of the anti-ICAM antibody within 6 hours after the onset of symptoms increased mortality, infarct volume and side effects (fever, infection) in patients with a worse functional outcome (108). One explanation for the failure of anti-ICAM antibody is the possibility that murine antibody would trigger an immunological response after administration. This hypothesis was further supported with animal studies showing that anti-ICAM-1 antibodies can increase selectin expression and activates complement, which in turn activates neutrophils (109,110). In a subsequent in vivo study in rats, anti-ICAM-1 antibodies administered prior to cerebral ischemia were found to sensitize the animals, resulting in larger infarcts, activation of complement system, and an upregulation of selectins (111). This experience with Enlimomab underscores the need to consider potentially harmful immune reactions to antibody administration and raises the possibility of improved success if humanized antibodies are employed.

Mac-1 blockade (Leukarrest, UK-279, 276)

Leukarrest (Hu23F2G) is a humanized, anti-Mac-1 (CD11b/18) antibody that had been shown to be effective in limiting infarct size, and neuronal damage following transient focal cerebral ischemia in rabbits (115). In a phase III clinical study, Leukarrest was tested in stroke patients however the trial was halted due to a failure to meet the predefined success criteria (112, 116).

UK-279, 276, a small recombinant glycoprotein that selectively binds to CD11b integrin of Mac-1 (CD11b/CD18), has been shown to be effective in reducing infarct size in reperfused animal models of stroke (100,113). In a phase 2 clinical study (Acute Stroke Therapy by Inhibition of Neutrophils; ASTIN) approximately 900 patients with ischemic stroke were

treated with UK-279, 276 within 6 h of stroke onset (114). In 204 patients UK-279, 276 was co administered with t-PA. No side effects were reported with UK-279, 276. The trial was terminated due to no improvement in outcome of stroke patients with UK-279, 276. However post-hoc analysis showed that there was a slight improvement in patients who received a combination therapy (rt-PA + UK-279, 276), indicating a possible beneficial effect of UK-279,279 in reperfusion injury related to t-PA administration.

CONCLUSIONS

The data generated from several experiments using cell surface adhesion molecules as targets of stroke therapy is promising yet inadequate. An ideal therapeutic agent that targets adhesion molecules for stroke treatment should not elicit an immune response, narrow the therapeutic window, and/or interfere with endogenous restorative processes. Unfortunately, existing reagents do not appear to fulfill all of these requirements.

The diversity of adhesion molecules used by different leukocyte populations to adhere to vascular endothelium warrants more attention as does the relative contributions of these different cell populations to ischemic brain injury. Monocytes and lymphocytes, for example, employ different adhesion glycoproteins than neutrophils to bind to vascular endothelium. While much effort has been devoted to defining the role of neutrophils to brain injury in ischemic stroke, emerging evidence suggests that mononuclear leukocytes play an equal or more important role in this injury process.

Adhesion molecule-directed therapies for stroke are based on the assumption that the recruitment of inflammatory/immune cells in postischemic brain is detrimental (117). However, some of these recruited cells may contribute to CNS regeneration following an ischemic stroke (41). For example, the results of a recent animal study indicate that activation of T-lymphocytes is neuroprotective in the setting of cerebral ischemia (118). Circulating lymphocytes, monocytes, and/or other circulating cells might induce a restorative phenotype following an ischemic insult via mechanisms that involve cell-to-endothelium contact. In a preliminary report (119), we have recently described the ability of selectin-specific antibodies to block the recruitment of exogenous bone marrow stromal cells (stem cells) into postischemic cerebral venules. Since stem cells may have therapeutic benefit in ischemic stroke, interfering with their recruitment or the trafficking of other restorative cells to the injured brain region may impair or delay the resolution of ischemia-induced tissue injury.

A promising, but inadequately studied, aspect of leukocyte adhesion-directed therapies is the combined use of anti-adhesion molecules with thrombolytic agents, such as tPA. The available evidence suggests the combination of rt-PA with a small molecule, non-immunogenic anti-adhesion agent may significantly extend the duration of the therapeutic window of rt-PA and confer some protection against brain injury. With improved and more readily available imaging methods for screening stroke patients and the development of novel thrombolytic agents, a larger proportion of stroke patients are likely to receive thrombolytic therapy in the future. A likely consequence of the increased frequency of aggressive intervention in stroke patients is an increased incidence of reperfusion-related brain pathophysiology that may be more responsive to anti-adhesion therapy. This possibility justifies a continued effort to more clearly define the role of adhesion molecules in the pathophysiology of cerebral ischemia-reperfusion and to develop novel reagents that effectively interfere with the adhesion of leukocytes to vascular endothelium.

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Ischemia-Reperfusion

NADPH oxidase

Notch-1

CD40-CD40L

Complement

Expression of cell
adhesion molecules

Recruitment of leukocytes
and platelets

↑ ROS production

Endothelial dysfunction

No-reflow
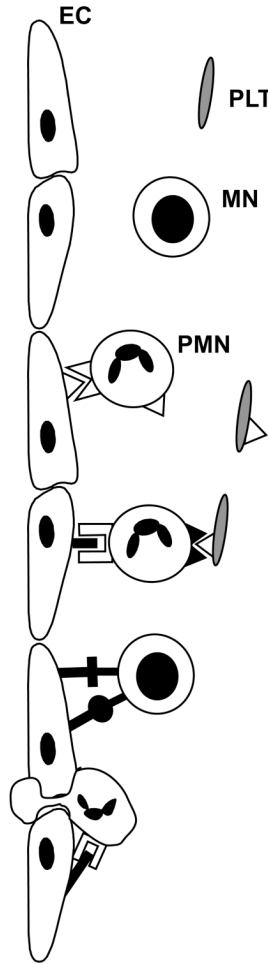
↑ Endothelial
restorative processes

EC

PLT

MN

PMN

Activation

Rolling

Adhesion

Transmigration

▷ Selectins

⊠ Sialyl-Lewis X

▬ ICAM-1

β-integrin

PSGL-1

MAC-1

LFA1

Figure 1.
Adhesion of leukocytes and platelets into cerebral microvessels following cerebral ischemia
reperfusion (I/R). I/R triggers activation of leukocytes and platelets and expression of adhesion
molecules on cerebral endothelium (EC). Initially polymorphonuclear cells (PMN) roll and
attach to endothelial cells. Platelets (PLT) adhere directly to endothelial cells or to PMN
adhering to cerebral endothelium. At late phases of reperfusion mononuclear cell (MN)
adhesion predominates. Adhesion of circulating cells further exacerbates the cerebral injury.

Table 1

Selected experimental studies targeting adhesion molecules in ischemic stroke.

Treatment	Model	Animal	Outcome	Reference
Nonspecific selectin antagonist	4 h of focal ischemia and 24 h of reperfusion	Rat	Improved neurological functions, decreased cerebral edema	70
Anti-L-selectin antibody	2 h focal ischemia 6 h of reperfusion	Rabbit	No reduction of infarct or neutrophil infiltration	71
P-selectin knockout	45 min ischemia 22 h reperfusion	Mice	Smaller infarct, reduced neutrophil influx, improved cerebral reflow	36
Anti-P-selectin antibodies	24 hr focal permanent ischemia	Rat	Reduced infarct size, brain water content and leukocyte infiltration	35
Anti-E-Selectin antibodies	45 min focal ischemia 24 h reperfusion	Mice	Reductions in neurological deficit, mortality, and infarct volumes	74
An analogue of Sialyl-LewisX (CY-1503)	2 h focal ischemia, 46 h reperfusion	Rat	Reduced infarct volume and neutrophil infiltration	30
Anti-P- and E-selectin	1 h focal ischemia, 72 h reperfusion	Nonhuman primate	Reduced infarct volume and neutrophil infiltration	75
Blocking selectins with synthetic oligopeptide	Permanent focal cerebral ischemia 24 h	Spontaneously hypertensive rats	Not effective	34
Mucosal tolerance to E-selectin	6 or 48 h permanent cerebral ischemia model	Spontaneously hypertensive stroke-prone rats	Reduction in infarct volume	78
ICAM-1 knockout animals	45 min focal cerebral ischemia followed by 22 h of reperfusion	Mice	Reduction in infarct volume and neurological deficit, increase in survival	38
Anti- ICAM-1 antibodies	Transient focal ischemia	Rat	Reduced infarct volume and neutrophil infiltration	88
Anti-Mac-1 antibodies	2 ischemia, 46 hours of reperfusion	Rat	Reduced infarct volume and neutrophil infiltration	91
Mice deficient in either LFA-1 or Mac-1	1 h focal ischemia 24 h of reperfusion	Mice	Reduced infarct volume, neurological deficit, leukocyte & platelet adhesion	50
tPA with anti-CD11b/CD18 antibodies	Embolic model	Rat	Better outcome with combination of agents	100