Monoclonal Antibodies Against ICAM-1 and CD18 Attenuate Cerebral Vasospasm After Experimental Subarachnoid Hemorrhage in Rabbits

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Background and Purpose—Inflammatory responses have been implicated in the elaboration of several forms of central nervous system injury, including cerebral vasospasm after subarachnoid hemorrhage (SAH). A critical event participating in such responses is the recruitment of circulating leukocytes into the inflammatory site. Two of the key adhesion molecules responsible for the attachment of leukocytes to endothelial cells are intercellular adhesion molecule-1 (ICAM-1) and the common β chain of the integrin superfamily (CD18). This study examined the effects of monoclonal antibodies on ICAM-1 and the effects of CD18 on cerebral vasospasm after SAH.

Methods—A rabbit model of SAH was utilized to test the influence of intracisternally administered antibodies to ICAM-1 and CD18 on cerebral vasospasm. Antibodies were administered alone or in combination, and the cross-sectional area of basilar arteries was assessed histologically on day 2 post-SAH.

Results—Treatment with antibodies to ICAM-1 or CD18 inhibited vasospasm by 22% and 27%, respectively. When administered together, the attenuation of vasospasm increased to 56%. All of these effects achieved statistical significance.

Conclusions—These findings provide the first evidence that the severity of cerebral vasospasm can be attenuated using monoclonal antibodies against ICAM-1 and CD18. The results reinforce the concept that cell-mediated inflammation plays an important role in cerebral vasospasm after SAH and suggest that therapeutic targeting of cellular adhesion molecules can be of benefit in treating cerebral vasospasm. (Stroke. 1998;29:1930-1936.)

Key Words: antigens ■ cell adhesion molecules ■ cerebral ischemia, transient ■ CD18 ■ ICAM-1 ■ subarachnoid hemorrhage ■ rabbits

Cell-mediated inflammation is a crucial event in tissue repair and restructuring after various forms of injury. However, increasing evidence indicates that cellular inflammation can also be quite detrimental to tissue recovery under certain circumstances. A critical role for cellular inflammation in the elaboration of tissue injury has been demonstrated in a wide range of acute and chronic pathophysiological conditions, including ischemia-reperfusion injury,1-9 rheumatoid arthritis,10 septic lung injury,11 atherosclerosis,12,13 septic shock,14 adult respiratory distress syndrome,14,15 allergic asthma,14 glomerulonephritis,14 ischemic renal injury,16 gastrointestinal system inflammation,14 multiple sclerosis,17-19 vasculitis,14,20,21 myocardial reperfusion injury,22,23 and graft rejection.14

Intracellular adhesion molecule-1 (ICAM-1) is a member of the immunoglobulin superfamily that is thought to be expressed on the endothelial surface in the early phase after tissue injury. ICAM-1 expression can be stimulated by various cytokines including lipopolysaccharides, tumor ne-

Received September 19, 1997; final revision received May 1, 1998; accepted May 13, 1998.
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vasospasm. Kubota et al.\textsuperscript{27} found that the peak ratio of T-helper to T-suppressor cells and the period of maximum macrophage infiltration occurred at 48 hours post-SAH. Ryba et al.\textsuperscript{28} reported IgM and C3 deposits in the endothelium of cerebral arteries after SAH in humans. Kasuya and Shimizu\textsuperscript{29} found activated complement components in the cerebral spinal fluid (CSF) of patients after SAH.

Using immunohistochemical techniques, Handa et al.\textsuperscript{30} recently demonstrated that the expression of ICAM-1 is increased in the endothelial cell layer of the rat basilar artery following SAH. Sills and colleagues\textsuperscript{31} demonstrated similar findings in a rat femoral artery model of vasospasm. Recently, we have documented elevated levels of ICAM-1 in the cerebrospinal fluid in humans within 48 hours of the time of SAH.\textsuperscript{32} Taken together, these findings are consistent with a role for cellular inflammation in hemorrhagic injury to cerebral blood vessels, and raise the possibility that targeting this event could be beneficial for the treatment of cerebral vasospasm after SAH.

The purpose of the present study was to evaluate the potential therapeutic value of targeting ICAM-1 or its ligand CD18 for the treatment of cerebral vasospasm after SAH. The protocol was designed to assess whether this leukocyte adhesion complex is upregulated merely as a by-product after SAH, or whether ICAM-1 and LFA-1 interactions provide a mechanistic bridge between the cytokine activation that occurs with SAH and the inflammatory component of vasospasm.

Materials and Methods

The experimental protocols used in this study were approved by the University of Virginia Animal Research Committee. Forty-two male New Zealand White rabbits weighing 3.3 to 3.7 kg were assigned randomly to 1 of 6 groups. Animals in group 1 served as controls and were not subjected to SAH (n=7). The animals in all other groups were subjected to experimental SAH as described below. Group 2 received experimental SAH without additional treatment (SAH-only; n=7). Group 3 was treated with an intracisternal injection of vehicle just before SAH (SAH+vehicle; n=7). Group 4 received an intracisternal injection of 10\textsuperscript{μ}g of anti-ICAM-1 before SAH (SAH+ICAM-1; n=7). Group 5 received an intracisternal injection of 10\textsuperscript{μ}g anti-CD18 before SAH (SAH+CD18; n=7). Group 6 was treated with an intracisternal injection of 10\textsuperscript{μ}g anti-ICAM-1 and 10\textsuperscript{μ}g anti-CD18 before SAH (SAH+ICAM-1&CD18; n=7). An additional group of animals (IgG; n=7), which was not part of the initial randomized design, received an intracisternal injection of 10\textsuperscript{μ}g mouse anti-human IgG nonspecific antibody. All injections were performed using a volume of 0.7 mL. All procedures were performed by 2 investigators (MB, A-LK) working in tandem and not blinded to the treatment group during surgery and euthanasia. Vascular measurements were performed in a blinded fashion.

Antibodies

The antibodies used in this study were anti-human ICAM-1 and anti-human CD18 (specific against the common \(\beta\) chain of the \(\beta_2\) subclass of the integrin superfamily of receptor proteins LFA-1, Mac-1, and p150–95). The lyophilized antibodies were purchased from R&D Systems. Both antibodies were of the mouse IgG1 subclass and have been shown to exhibit good cross-reactivity with rabbit cells.\textsuperscript{23,29,30,32} Because of the prohibitive costs of administering the antibodies systemically, in the present study we used intracisternal injections to limit the amount of antibodies required. The antibodies were reconstituted with distilled water and a dose of 10\textsuperscript{μ}g per antibody per animal was selected to achieve an initial concentration in the CSF of approximately 1.25\textsuperscript{μ}g antibodies per mL of CSF. This dose was selected with the goal of saturating the available ICAM-1– and CD18-binding sites. The calculation of this dose was based on a previous study\textsuperscript{1} in which ICAM antibody was administered systemically to rabbits; the final doses were adjusted on the basis of the estimated blood and CSF volumes of the rabbits used in this study. The anti-IgG was obtained from Sigma Chemical Co and was reconstituted as described above to provide a dose of 1.25\textsuperscript{μ}g antibodies per mL of CSF.

Induction of Experimental SAH

All animals subjected to experimental SAH were anesthetized by intramuscular injection of a mixture of ketamine (Ketaset, 50 mg/kg) and xylazine (Rompun, 10 mg/kg), paralyzed with pancuronium bromide (0.08 mg/kg), intubated, and ventilated with a Harvard model 683 dual-phase ventilator (Harvard Apparatus Co). A 23-gauge butterfly needle was inserted percutaneously into the cisterna magna. After withdrawal of 1.0 mL of CSF, drug or placebo was given followed by an interval of 1 minute to allow diffusion of the antibody; subsequently, 3 mL of nonheparinized blood from the central ear artery was injected into the subarachnoid space. The animals were then placed in a head-down position for 15 minutes to hold the blood in the basal cisterns. Arterial blood gases were analyzed during the surgical procedure and maintained within the physiological range. After recovering from anesthesia, the rabbits were observed for possible neurological deficits and then returned to the vivarium. The combination of ketamine and xylazine was selected on the basis of reports of its efficacy for analgesia and anesthesia.\textsuperscript{35,36}

Perfusion-Fixation

All animals subjected to experimental SAH were euthanized by perfusion-fixation 48 hours after SAH induction. The animals were anesthetized, intubated, and ventilated as described above. The ear artery was catheterized for monitoring blood pressure and for blood gas analysis. When satisfactory respiratory parameters were obtained, a thoracotomy was performed, the left ventricle cannulated, the right atrium opened widely, and the abdominal aorta clamped. After perfusion of a flushing solution (Hanks’ balanced salt solution [Sigma Chemical Co], pH 7.4, at 37°C, 300 mL), fixative was perfused (2% paraformaldehyde, 2% glutaraldehyde in Hanks’ balanced salt solution, pH 7.4, at 37°C, 200 mL). Perfusion was performed at a standard height of 100 cm from the chest. Animals in the control group were killed using the same procedure. Brains were then removed and stored in fixative at 4°C overnight.

Embedding, Morphometry, and Statistical Analysis

Basilar arteries were dissected from the brain and arterial segments from the proximal third of the artery were cut, with care being taken to avoid branching points. The vessels were embedded in epoxy resin, and cross-sections were cut at a thickness of 0.5 \(\mu\)m. The sections were mounted onto glass slides and stained with 0.5% toluidine blue for light microscopic analysis. The vessels were measured using computer-assisted morphometry (Image 1, Universal Imaging Corp.). Automated measurements of the cross-sectional area of the arterial sections were taken by an investigator who was blinded to the identity of the group to which the animals belonged. Five cross-sections of each vessel were selected randomly for measurement, and the average of these measurements was calculated. Statistical comparisons were performed using a Kruskal-Wallis 1-way ANOVA with Dunnett’s test. Statistical significance was accepted at \(P<0.05\).

Results

The physiological parameters measured before perfusion in groups 1–6 are listed in Table 1. There were no significant differences among the groups in terms of body weight, arterial pH, PO\textsubscript{2}, PCO\textsubscript{2}, or mean arterial blood pressure. On removal of the brain from the skull, a thick subarachnoid clot was present, covering the surface of the brain stem in all animals subjected to experimental SAH. There were no
apparent differences in the size of the subarachnoid clots observed in the animals from the different treatment groups. This finding suggests that the adhesion molecule antibodies do not attenuate the blood clot obtained after the injection of autologous blood.

Qualitative light microscopic examination of the basilar arteries in the SAH-only and SAH+vehicle groups revealed substantial corrugation of the internal elastic lamina, whereas arterial corrugation was less prominent in animals treated with the antibodies (Fig 1). The group values obtained from the quantitative measurements of the cross-sectional areas of the basilar arteries are shown in Fig 2. The SAH-only and SAH+vehicle groups exhibited large and statistically significant reductions in arterial area when compared with the control group. The values for the SAH-only and SAH+vehicle groups did not differ significantly. The magnitude of cerebral vasospasm was significantly attenuated in animals treated with antibodies against the adhesion molecules. Treatment with anti–ICAM-1 or anti-CD18 reduced the degree of arterial spasm by 22% and 27%, respectively, when compared with the SAH+vehicle group. Combined treatment with the 2 antibodies resulted in a substantial and significant attenuation of the vasospastic response (ie, a 56% reduction in vasospasm). Although this protective effect was greater than that of either individual antibody, the differences between the combined antibody treatment and the individual antibody treatments did not achieve statistical significance. Treatment with an anti-IgG antibody alone did not reduce vasospasm (Fig 2).

**Discussion**

A role for cellular inflammation in the pathophysiology of cerebrovascular disease has gained increasing support in recent years. Most of the experimental efforts in this area have focused on the role of polymorphonuclear leukocytes (PMNLs) and other invading cells in aggravating neuronal injury after ischemia.1,7,37 The importance of this mechanism is underscored by studies demonstrating that antibodies and synthetic peptides that block cellular adhesion are capable of limiting cerebral ischemic injury.1,3,7,8,38,39 Antibodies against ICAM-1 have been found to reduce infarct volumes in experimental stroke models.1,3 Connolly et al3 have shown that homozygous ICAM-1 knockout mice demonstrated a 3.7-fold reduction in infarct volume compared with control animals. Agents that inhibit the recognition and adherence of PMNLs to the cerebrovasculature may therefore prove to be valuable in the treatment of stroke.

![Figure 1](http://stroke.ahajournals.org/)

**Figure 1.** Effect of antibody treatment on basilar arteries subjected to SAH. Cross-sections of basilar arteries are shown from the control (A), SAH-only (B), and SAH+CD18 and ICAM-1 antibodies (C) groups. The SAH-only vessel exhibits severe narrowing and corrugation of the internal elastic lamina (B). In contrast, vascular narrowing and corrugation are less pronounced in the vessel from the antibody-treated group (C). Calibration bar=0.1 mm.
In addition to its role in mediating ischemic injury to the brain parenchyma, it is plausible that cellular inflammation could contribute directly to the dysfunction of large cerebral vessels after intracranial hemorrhage. The primary cause of morbidity and mortality after SAH is cerebral vasospasm. This delayed spastic response provides a secondary ischemic challenge to the brain that is often devastating in the wake of ruptured aneurysmal bleeding. As discussed in the introduction to this article, recent evidence suggests that cellular inflammation can occur after SAH, both as an immune complex deposition and as a cellular infiltrate. How this pathway is initiated remains unclear, but the ICAM-1/LFA-1 complex is a potential mediator.

ICAM-1 theoretically holds potential as a mediator of inflammation and vasospasm. ICAM-1 can be induced by cytokines (such as interleukin-1, tumor necrosis factor-α, and lipopolysaccharides) on the cerebral endothelial surface, serving as a ligand for the integrin CD18 or LFA-1 present on leukocytes. Cytokines are known to be produced in hypoxic/ischemic endothelial cells and have been detected in the spinal fluid of patients with SAH. Rieckmann and colleagues demonstrated that a soluble form of ICAM-1 attenuated this response, suggesting a capacity for negative feedback of the cytokine-induced upregulation of ICAM-1. Therapies targeting ICAM-1 could be of value in the management of vasospasm after SAH for 2 reasons. First, blocking the function of ICAM-1 may provide cellular protection against ischemia. Second, ICAM-1 may serve as an important component in the pathogenesis of vasospasm, acting to promote leukocyte migration across the vascular endothelium and initiating inflammation in the vascular wall that may contribute to vasospasm. Clinical efforts to target this inflammatory component of vasospasm have focused on the general immunosuppressant cyclosporine, which has been shown to reduce vasospasm in a canine model but has demonstrated mixed results in human trials. The therapeutic value of general immunosuppression using cyclosporine thus remains somewhat controversial.

Limitations of the Model
Substantial additional preclinical and clinical work will obviously be necessary to elucidate the possible therapeutic value of targeting adhesion molecules for the treatment of cerebral vasospasm. Several key issues remain to be addressed regarding the dosing regimen, timing of treatment, and avenue of administration of the antibodies that were used in this study. An optimal treatment for vasospasm should be effective when administered systemically after SAH at doses that produce minimal side effects. It remains to be determined whether adhesion molecule antibodies will be effective when treatment is initiated subsequent to SAH or if penetration through the blood-brain barrier (BBB) will be possible. It is feasible for certain large molecules such as IgG antibodies to cross the BBB after SAH. Sasaki et al showed transient BBB leakage at the time of experimental SAH with extensive disturbance of the BBB seen thereafter, via opening of the interendothelial junctions. Johshita and colleagues replicated these findings in the rabbit model used in the present study. This particular model was used for several reasons. The rabbit basilar artery model is a simple and inexpensive one in which the vessels undergo a delayed, progressive, reproducible reduction in size that has been shown to be refractory to traditional vasodilators.

Another important consideration when evaluating the present study is determining whether the doses or the particular combination of antibodies selected for the present study provided a maximal protective effect. It is conceivable that a modification of the doses or combination of antibodies could further enhance their protective actions. Although an apparent enhancement of the protective effect was obtained when both antibodies were delivered in combination, the current results do not permit conclusions concerning possible additive effects of targeting multiple components of the adhesion response. It is conceivable that targeting both the endothelial adhesion molecule induced by SAH through cytokine activation (ICAM-1) and its ligand present on circulating leukocytes...
(CD18) could have an additive effect. Conversely, the enhanced protection could reflect the possibility that the dose of a given antibody did not achieve a maximal effect. This is an avenue for further study. Finally, the specific antibodies used were available commercially (R&D Systems), but the specific epitope targeted by the antibodies is not known, providing a further hurdle to any clinical relevance of these findings.

The present study did not determine the bioavailability of the antibodies. Sills and colleagues have determined that ICAM-1 is present as early as 3 hours from the time of SAH. It is conceivable that cellular adhesion is an important step in the initiation of vasospasm, but it is not necessarily important throughout the entire duration of the phenomenon. Further studies will be required to determine the optimum duration of treatment. It is also unclear whether systemic application of adhesion molecule antibodies can provide an effective method to limit cerebral vasospasm. It is conceivable that adhesion molecule antibodies will turn out to be an inefficient means of suppressing cellular adhesion in terms of their pharmacokinetic and economic limitations. Antibodies possess inherent constraints with respect to their modifiability, bioavailability, and structure-activity profiles. Moreover, it is currently expensive and time-consuming to produce selective antibodies against the adhesion molecules involved in PMNL adherence. In contrast, modifiable synthetic compounds that block cell adhesion, such as fibronectin peptides or specific peptide inhibitors of ICAM-1 and LFA-1, can be developed to provide more desirable pharmacokinetic and functional characteristics and to provide a more cost-effective alternative to the use of antibodies. Despite the possible limitations of therapies using antibodies against adhesion molecules, it is important to stress that encouraging results have been obtained with the systemic administration of such antibodies for the treatment of experimental cerebral ischemia. It is therefore reasonable to have guarded optimism about the potential therapeutic value of adhesion molecule antibodies for the treatment of vasospasm.

Conclusions

In summary, the results from the present study indicate that antibodies directed against adhesion molecules participating in PMNL-endothelium adherence are capable of attenuating cerebral vasospasm after SAH. These findings provide the first evidence for a role of the ICAM-1/LFA-1 interaction in the pathogenesis of cerebral vasospasm. Although these findings are preliminary, they provide encouragement that inhibitors of cellular inflammation may prove a novel clinical avenue for the treatment of cerebral vasospasm.

Acknowledgments

This work was supported in part by NIH grant HL49396 to Dr Lee. We appreciate the assistance of Sarah B. Hudson and Jennifer L. Collins for their help in preparing the manuscript.

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**Editorial Comment**

The authors have shown that antibodies to ICAM-1 and the common β chain of the integrin superfamily (CD18) decreased vasospasm by 22% and 27%, respectively, in a rabbit model of SAH. The combination of the 2 antibodies together had an even more marked preventive effect, decreasing vasospasm by 56%. I have several comments and also pose questions that these experiments raise and that will need to be answered in additional experiments. Implicit in the use of anti-inflammatory treatments for vasospasm is the hypothesis that inflammation somehow leads to arterial narrowing. Patients with meningitis, which must be regarded as the ultimate form of subarachnoid inflammation, do develop arterial narrowings and infarction, but it is not pathologically the same as vasospasm after SAH. Patients with meningitis usually have increased cerebral blood flow in the acute phase. Until this discrepancy can be explained, the role of inflammation in vasospasm will be questioned. Furthermore, there is no mechanism for how preventing leukocytes from entering the subarachnoid space prevents vasospasm. It is speculative to suggest that this might, for example, decrease erythrocyte lysis and allow clot clearance by natural fibrinolysis before hemolysis occurs. Sills and colleagues showed that vasospasm of the rat femoral artery was associated with upregulation of ICAM-1 and with inflammatory cell infiltration. They suggested that these events damaged endothelial cells and promoted arterial wall remodeling, both of which contributed to vasospasm. The therapeutic results of potent anti-inflammatory medications such as cyclosporin A have not been marked. Additional tests of this treatment strategy are required in higher level species.

There is always some concern about the relevance of arterial narrowing 2 days after subarachnoid blood injection to vasospasm in humans. In primates, significant vasospasm does not develop until 3 or 4 days after an SAH produced by...
placement of blood clot in the subarachnoid space. It is not clear whether the pathogenetic processes are the same but occur on a different time scale or whether the pathogenesis is different in different species. In our experience and from review of photomicrographs from models of vasospasm of systemic arteries, there seems to be much more inflammation than in intracranial blood clot placement models. This suggests that there is interspecies variability in the role of various pathogenetic processes.

How did antibodies administered intracisternally affect a process of leukocyte adhesion that occurs primarily at the blood-endothelial cell interface? If this treatment was to be used clinically, it would seem to be ideal to administer the antibodies systemically, so that they would be present in the blood with the leukocytes and endothelial cells on which the receptors to be blocked reside. It is interesting but difficult to explain that an additive effect was observed when the 2 antibodies were administered together. The epitopes against which the antibodies act are unknown, and this always raises the question as to whether there are cross-reactivities with other receptors that could mediate the observed effects. The use of nonspecific mouse anti-human IgG seems to rule out a nonspecific effect of mouse antibodies against vasospasm in rabbits.

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Stroke. 1998;29:1930-1936
doi: 10.1161/01.STR.29.9.1930
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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