Prevention of vasospasm following subarachnoid hemorrhage in rabbits by anti-CD11/CD18 monoclonal antibody therapy

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Object. Adhesion of leukocytes and their migration into the periadventitial space may be critical in the pathophysiology of vasospasm following subarachnoid hemorrhage (SAH). The cell adhesion molecules involved in this process are lymphocyte function–associated antigen–1 (CD11a/CD18) and macrophage antigen–1 (CD11b/CD18), which are present on neutrophils/macrophages, and intercellular adhesion molecule–1 (CD54), which is present in endothelial cells. A humanized monoclonal antibody (mAb), Hu23F2G, targets CD11/CD18 and prevents leukocyte adhesion to endothelial cells. In this study, systemic administration of Hu23F2G prevented vasospasm in the rabbit model of SAH.

Methods. Twenty-six New Zealand White rabbits were injected with autologous blood into the cisterna magna to induce SAH, after which they were randomized to receive injections of either Hu23F2G (10 animals) or a placebo at 30 minutes and 24 and 48 hours after SAH (six animals). Control animals underwent sham operations (four animals) or SAH alone (six animals). The animals were killed 72 hours after SAH, their bodies perfused and fixed, and their basilar arteries processed for morphometric analysis. Peripheral white blood cells (WBCs) were counted at 72 hours. The percentages of luminal patency were compared using the Student t-test. The presence of neutrophils and macrophages was confirmed by immunohistochemical analysis in which a rat anti–rabbit anti-CD18 mAb and cresyl violet were used.

Treatment with Hu23F2G resulted in the significant prevention of vasospasm. Animals treated with Hu23F2G had 90 ± 7% lumen patency compared with 65 ± 7% in the placebo group (p = 0.025). The percentage of lumen patency in the SAH-only group was 59 ± 10%. The mean WBC count was 16,300 ± 2710/µl in the treatment group, compared with 7000 ± 386/µl in the control group (p = 0.02). Administration of Hu23F2G produced increased numbers of WBCs in 70% of the animals treated.

Conclusions. This study supports the concept that leukocyte–endothelial cell interactions play an important role in the pathophysiology of chronic vasospasm after SAH. Systemic therapy with an anti-CD11/CD18 mAb prevents vasospasm after SAH by inhibiting adhesion of neutrophils and macrophages and their migration into the periadventitial space.

Key Words • vasospasm • subarachnoid hemorrhage • leukocyte–endothelial cell interaction • anti-CD11/CD18 monoclonal antibody • rabbit
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...modation of leukocytes into the periadventitial space. Preliminary studies in a nonhuman primate model of SAH indicate that Hu23F2G might be of benefit in the treatment of vasospasm. 

...We and others have previously shown that blocking leukocyte–endothelial cell interactions with mAbs directed against CAMs prevents posthemorrhagic vasospasm in rats, rabbits, and monkeys. Systemic therapy with an anti–ICAM-1 mAb prevented chronic vasospasm and decreased the number of periadventitial macrophages and neutrophils in the rat femoral artery model of vasospasm. Treatment with anti–ICAM-1 and anti–LFA-1 mAbs also prevented chronic vasospasm and decreased the number of periadventitial inflammatory cells in rats. Antibodies against ICAM-1 and CD18 administered intrathecally prevented vasospasm in a rabbit model as well. Similarly, ibuprofen, which inhibits the endothelial expression of ICAM-1 and VCAM-1, prevented vasospasm after SAH in dogs and in the femoral artery model of vasospasm in rats.

We hypothesized that the systemic administration of an anti–CD11/CD18 mAb, which could block the adhesion of neutrophils and macrophages and their migration into the periadventitial space, prevents vasospasm after SAH. In this study we describe the effect of systemic therapy with Hu23F2G on the lumen patency of the BA and on the number of peripheral WBCs in the rabbit model of vasospasm after SAH.

Materials and Methods

Experimental Design

Animals were randomized to four experimental groups. Animals in the first group (sham-operated group, four animals) underwent surgery with exposure of the dura mater only and without CSF extraction or a cisternal injection of blood. Animals in the second group (SAH-only group, six animals) underwent injection of 1.5 to 2 ml of autologous arterial blood into the cisterna magna to create an SAH. Animals in the third (SAH + Hu23F2G group, 10 animals) and fourth (SAH + placebo group, six animals) groups underwent intraperitoneal injections of either Hu23F2G or vehicle at 30 minutes and 24 and 48 hours after blood injection. The rabbits were killed at 72 hours post-SAH, when peak vasospasm occurs in rabbits as previously determined by our group and others. Animals were therefore killed 72 hours after induction of SAH is present 72 hours after blood injection into the cisterna magna. Animals were therefore killed 72 hours after induction of experimental SAH by an intraperitoneal injection of sodium pentobarbital (200 mg/kg), after which in situ perfusion fixation was performed. The animals were anesthetized as described earlier, a mid-sternal thoracotomy was performed, the right atrium was pierced for exsanguination, and the left ventricle was cannulated. Transcardiac perfusion was performed with 300 ml of a 0.1-M phosphate-buffered saline solution followed by 500 ml of ice-cold 4% paraformaldehyde, which was delivered by a peristaltic pump at 100 rpm (25 ml/minute) (Watson-Maulden-Bredel, Falmouth, UK). The BA and the brainstem were harvested en bloc and immersed in a 0.1-M phosphate buffer–20% sucrose solution at 4°C for 3 days for cryoprotection. Specimens were snap-frozen in −60°C methylbutane and stored at −80°C. Transverse sections (20-μm) were obtained with a microtome cryostat at 200-μm intervals beginning at the termination of the BA. Tissue slices were mounted on Superfrost Plus slides (Fisher Scientific Co., Pittsburgh, PA) for H & E staining. Additional staining of BA cross-sections (four per group) was performed using a rat anti–rabbit CD18 antibody and cresyl violet to detect the periadventitial localization of CD18-positive cells.

Histological Assessment

Angiographic evidence of peak vasospasm in the rabbit model of SAH is present 72 hours after blood injection into the cisterna magna. Animals were therefore killed 72 hours after induction of experimental SAH by an intraperitoneal injection of sodium pentobarbital (200 mg/kg), after which in situ perfusion fixation was performed. The animals were anesthetized as described earlier, a mid-sternal thoracotomy was performed, the right atrium was pierced for exsanguination, and the left ventricle was cannulated. Transcardiac perfusion was performed with 300 ml of a 0.1-M phosphate-buffered saline solution followed by 500 ml of ice-cold 4% paraformaldehyde, which was delivered by a peristaltic pump at 100 rpm (25 ml/minute) (Watson-Maulden-Bredel, Falmouth, UK). The BA and the brainstem were harvested en bloc and immersed in a 0.1-M phosphate buffer–20% sucrose solution at 4°C for 3 days for cryoprotection. Specimens were snap-frozen in −60°C methylbutane and stored at −80°C. Transverse sections (20-μm) were obtained with a microtome cryostat at 200-μm intervals beginning at the termination of the BA. Tissue slices were mounted on Superfrost Plus slides (Fisher Scientific Co., Pittsburgh, PA) for H & E staining. Additional staining of BA cross-sections (four per group) was performed using a rat anti–rabbit CD18 antibody and cresyl violet to detect the periadventitial localization of CD18-positive cells.

Morphometric Analysis

Luminal cross-sectional areas were outlined and the circumference of the BA was measured by performing computerized analysis (MCID; Imaging Research, St. Catharines, ON, Canada). Six sections of each BA (each section 20 μm thick and obtained 200 μm apart) were evaluated and averaged to control for vessel deformation and off-transverse sections. The vessel perimeter was obtained by interactive measurements of vessel sections. Estimated cross-sectional areas were converted to lumen-patency percentages and absolute values were defined by the average of cross-sectional areas from sham-operated animals.

Statistical Analysis

Vessel perimeters and WBC counts are expressed as mean values ± standard errors of the means. Mean perimeters of the BAs are expressed as percentages of lumen patency obtained by dividing the mean perimeter of each group by the mean perimeter of the sham-operated group. Mean vessel perimeters (in millimeters) were compared using the Student t-test. A probability value less than 0.05 was considered significant. Statistical analysis was performed using SPSS Version 8.0 for Windows (SPSS, Inc., Chicago, IL).
Results

Treatment with Hu23F2G resulted in a significant increase in BA lumen patency as well as in the peripheral WBC counts. Whereas the lumen patency of the SAH + Hu23F2G group was 90 ± 7%, that of the SAH + placebo group was 65 ± 7% (p = 0.025) (Fig. 1). By comparison the lumen patency of the SAH-only group was 59 ± 10%. Similarly, although the peripheral WBC count of the SAH + Hu23F2G group was 16,300 ± 2710/µl, that of the SAH + placebo group was 7000 ± 386/µl (p = 0.02; Fig. 2). It was noted that within the SAH + Hu23F2G group seven of 10 animals had elevated peripheral WBC counts, but three did not. The mean peripheral WBC counts in animals whose response was an elevated count was 20,517 ± 2371/µl, compared with 6310 ± 505/µl in animals without elevated WBC counts (p = 0.005). Qualitative observations of immunohistochemical tests for CD18 showed a decrease in CD18-positive cells in the periadventitial space of BAs from rabbits treated with Hu23F2G when compared with the periadventitial space of BAs from animals treated with placebo (Fig. 3).

Discussion

In this study we describe the systemic administration of a humanized anti-CD11/CD18 mAb for the treatment of vasospasm in a rabbit model of SAH. We found that treatment with Hu23F2G resulted in increased lumen patencies, prevention of morphometric vasospasm, and increased numbers of peripheral WBCs. The Hu23F2G antibody binds to β-2 integrins including LFA-1 and Mac-1, which are expressed on the surface of macrophages and neutrophils. Such binding prevents their interaction with ICAM-1 and, thus, their adhesion to the endothelial surface. The biological effect of Hu23F2G was confirmed by an increase in peripheral WBCs at 72 hours after SAH, which was observed in 70% of the treated animals.

The Hu23F2G antibody has been used previously in New Zealand White rabbits, nonhuman primates, and humans. A reduced likelihood of immunogenic reactions has been achieved by humanizing the antibody, which markedly decreases the likelihood of a human anti–mouse antibody type reaction and maintains the binding efficacy of the murine precursors. Whereas a human anti–mouse antibody response has been identified in 20 to 40% of patients treated with murine antibodies, such a response has been identified in only 7% of patients treated with humanized antibodies.6 The Hu23F2G antibody uses the human IgG4 heavy chain, which has a decreased fixation of complement and low binding of Fc receptors.27,52

Upregulation of ICAM-1 and other CAMs on the endothelial surface following aneurysmal SAH promotes binding of macrophages and neutrophils through LFA-1 and Mac-1, thus creating a determinant event in the pathogenesis of posthemorrhagic vasospasm.15 After aneurysmal SAH, erythrocytes in the subarachnoid space cluster around the vessel wall as a thrombus. Acute-phase reactants such as interleukin-1β,9 tumor necrosis factor–α,1 and interferon-γ,24 among others, are produced in the thrombus and induce upregulation of selectins and ICAM-1 in the endothelial layer.42 Upregulation of ICAM-1 causes the arrest of rolling leukocytes, adhesion, and diapedesis into the subarachnoid space.27 Macrophages and neutrophils in the subarachnoid space are then attracted into the periadventitial thrombus by released chemoattractants. These leukocytes proceed to phagocytose erythrocytes and debris. After phagocytosis, however, the leukocytes die in the subarachnoid space and release ETs,11,20 O₂ free radicals,43 chemo-
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kines,21 and other products,7,15,20,42 which damage the endothelium, decrease the synthesis of endothelium-derived NO, and cause chronic vasospasm.

The rabbit model of SAH was first described by Offerhaus and van Gool17 in 1969 to analyze electrocardiographic changes after SAH. Nevertheless, the most common type of rabbit model used to induce vasospasm after SAH is the one described by Chan and associates10 in which SAH is created by injection of arterial blood into the cisterna magna.28 This method has achieved excellent correlation between angiographic vasospasm and morphometric measurements of perfusion-fixed cross-sections of the BA.31 Injection of blood into the cisterna magna results in histopathological changes after SAH,10,22. In our series we had a mortality rate of less than 10%. Disadvantages of this model include the development of subacute vasospasm (on Day 3 in comparison with the human disease in which vasospasm occurs 7–10 days after SAH) and the absence of neurological deficits after vasospasm; however, the latter is true for all animal models of posthemorrhagic vasospasm.28,31

The inflammatory CAM hypothesis of vasospasm after SAH can be reconciled with the extensive evidence that shows the important role of ETs and NO depletion in vasospasm. Macrophages and neutrophils in the subarachnoid space release vasoplastic molecules, particularly ET-1.2,7,20,42 Endothelin-1, the most common isoform of ET, is a potent vasoplastic molecule involved in posthemorrhagic vasospasm. In addition, ET-1 is also secreted by endothelial cells, neurons, and astrocytes.7,20 Endothelin-1 binds primarily to the ETα receptor located mainly in smooth-muscle cells and activates a Gs protein that controls voltage-dependent Ca channels, resulting in vasoconstriction.11 Simultaneously, ET-1 contributes to the decreased synthesis of NO, impairing NO-dependent vasodilation.2 After neutrophils and macrophages degranulate, free radicals such as OH− and O2− are released into the periadventitial space and eliminate endothelium-dependent relaxation2 through destruction of endogenous NO. These free radicals also cause lipid peroxidation, enzymatic inhibition, and elevation of Ca++ among other events.35 which damage the endothelium and contribute to the development of vasospasm.

Conclusions

Treatment with Hu23F2G, which disrupts interactions between endothelial ICAM-1 and the leukocyte integrins LFA-1 and Mac-1, and thus could prevent the migration of neutrophils and macrophages into the periadventitial space, prevents morphometric vasospasm after SAH in rabbits. These findings support the role of leukocyte–endothelial cell interactions in the pathogenesis of chronic posthemorrhagic vasospasm.

Acknowledgment

This work was supported in part by a generous gift from Mr. and Mrs. J. Dorsey Brown to Dr. Tamargo.

References


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