Association between cerebrospinal fluid levels of asymmetric dimethyl-L-arginine, an endogenous inhibitor of endothelial nitric oxide synthase, and cerebral vasospasm in a primate model of subarachnoid hemorrhage

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Object. Decreased availability of nitric oxide (NO) has been proposed to evoke delayed cerebral vasospasm after subarachnoid hemorrhage (SAH). Asymmetric dimethyl-L-arginine (ADMA) inhibits endothelial NO synthase (eNOS) and, therefore, may be responsible for decreased NO availability in cases of cerebral vasospasm. The goal of this study was to determine whether ADMA levels are associated with cerebral vasospasm in a primate model of SAH.

Methods. Twenty-two cynomolgus monkeys (six control animals and 16 with SAH) were used in this study. The levels of ADMA, L-arginine, L-citrulline, nitrates, and nitrites in cerebrospinal fluid (CSF) and serum were determined on Days 0, 7, 14, and 21 following onset of SAH. Cerebral arteriography was performed to assess the degree of vasospasm.

Western blot analyses of the right and left middle cerebral arteries (MCAs) were performed to assess the expression of eNOS, type I protein–arginine methyl transferase (PRMT1) and dimethylarginine dimethylaminohydrolase (DDAH2).

Cerebrospinal fluid levels of ADMA remained unchanged in the control group (six animals) and in animals with SAH that did not have vasospasm (five animals; p = 0.17), but the levels increased in animals with vasospasm (11 animals) on Day 7 post-SAH (p < 0.01) and decreased on Days 14 through 21 (p < 0.05). Cerebrospinal fluid levels of ADMA correlated directly with the degree of vasospasm (correlation coefficient = 0.7, p = 0.0001; 95% confidence interval: 0.43–0.83). Levels of nitrite and nitrate as well as those of L-citrulline in CSF were decreased in animals with vasospasm. Furthermore, DDAH2 expression was attenuated in the right spastic MCA on Day 7 post-SAH, whereas eNOS and PRMT1 expression remained unchanged.

Conclusions. Changes in the CSF levels of ADMA are associated with the development and resolution of vasospasm found on arteriograms after SAH. The results indicate that endogenous inhibition of eNOS by ADMA may be involved in the development of delayed cerebral vasospasm. Inhibition of ADMA production may provide a new therapeutic approach for cerebral vasospasm after SAH.

KEY WORDS • subarachnoid hemorrhage • nitric oxide • nitric oxide synthase • asymmetric dimethyl-L-arginine • vasospasm • Macaca fascicularis

D ESPITE extensive experimental and clinical research during the last five decades, delayed cerebral vasospasm, a constriction of the conductive cerebral arteries following SAH that leads to delayed ischemic neurological deficits, continues to be feared complication and a major cause of morbidity and mortality in patients who suffer SAH.14,28

There is growing evidence that cerebral vasospasm is associated with decreased availability of NO in the cerebral vessels.16,26,32 Nitric oxide, a powerful vasodilator, plays a major role in regulating cerebrovascular tone.10,26,36,37 Nitric oxide is synthesized by a NOS family and is derived from the terminal guanidino nitrogen atoms of L-arginine. After synthesis by eNOS, NO diffuses across the endothelial cell membrane, enters vascular smooth-muscle cells, and activates soluble guanylate cyclase, which produces cyclic guanosine-3’,5-monophosphate, mediating vasodilation.31,39 Production of NO can be endogenously blocked by ADMA, a competitive inhibitor of NOS.40,41 We hypothesized that decreased NO availability in the setting of vasospasm after SAH may be evoked by ADMA.
Asymmetric dimethyl-L-arginine in vasospasm after SAH

The goals of this study were the following: 1) determine whether ADMA levels are detectable in CSF and serum; 2) establish whether time-dependent changes in ADMA levels are associated with the development and resolution of vasospasm in a primate model of delayed cerebral vasospasm after SAH; 3) examine the levels of NO metabolites in CSF; and 4) examine the expression of enzymes involved in NO production and ADMA metabolism in the MCAs.

Material and Methods

The animal protocol was reviewed by the National Institute of Neurological Disorders and Stroke Animal Care and Use Committee and met the NIH guidelines for animal care.

Model of Cerebral Vasospasm

Cynomolgus monkeys were prepared using a well-established primate model of delayed vasospasm following SAH.1,4 After induction of general anesthesia, the monkeys underwent right frontotemporal craniectomy under aseptic conditions. The arachnoid of the right sylvian fissure was dissected. The proximal 1.4 cm of the right MCA was exposed and 5 ml of preclotted autologous arterial blood or 5 ml of normal saline (sham surgery) was placed around the artery. The dura mater and wound were closed in layers. After their recovery of the gag reflex, the monkeys were extubated. The animals were observed until Days 7, 14, or 21 post-SAH and were killed at the end of the experiment.

Animal Preparation

Twenty-two cynomolgus monkeys were used in this study. Three sham-operated animals (observed until Day 7 after surgery) and three naive animals were combined in one control group (six animals) because no differences in physiological parameters or results were observed in these two groups. Sixteen animals with induced SAH were killed according to the following schedule: Day 7 (seven animals), Day 14 (four animals), and Day 21 (five animals). Both the right and left MCAs in these monkeys were harvested and snap frozen for later Western blot analyses.

Arteriographic Studies

To assess the degree of vasospasm, baseline cerebral arteriography was performed preoperatively on Day 0, and again on Days 7, 14, or 21 after induction of SAH, as described elsewhere.1 At least two arteriograms were obtained in each animal, one image before SAH and the second on Day 7 post-SAH. Arteriography was performed while the monkey was in the state of general anesthesia induced by a combination of 0.5% isoflurane–pancuronium and no endotracheal intubation. Systemic arterial blood pressure and end expiratory PCO2 in the monkeys were continuously monitored and remained stable throughout the procedure. A femoral catheter cutoff was performed under aseptic conditions and a No. 3 French polyethylene catheter was advanced under fluoroscopic control to the right internal carotid artery. Between 0.75 and 1 ml of contrast medium (RenoCal-76 [diatrizoate meglumine and diatrizoate sodium]) was injected into the catheter by hand. Subtraction images of the anterior–posterior projection were acquired. The area of the proximal 14-mm segment of the right MCA was measured by three blinded examiners using a computerized image analysis system, as previously described.1 The arteriographic pressure of vasospasm was quantitated relative to findings on the baseline arteriograms of each animal and was classified in the following manner: no vasospasm (< 11%) or a mild (11–25%), moderate (26–50%), or severe (> 50%) reduction in the area of the right MCA in the anteroposterior view of the arteriogram after SAH.

Sample Collection

Samples of CSF and serum were collected on Day 0, at the time of surgery, and at the time of arteriography on Days 7, 14, and 21 post-SAH, after general anesthesia had been induced by administration of a 0.5% isoflurane–pancuronium mixture. The samples of CSF and serum were immediately centrifuged, following which the supernatants were snap frozen and stored at −80°C.

High-Performance Liquid Chromatography

High-performance liquid chromatography was performed to detect the levels of free ADMA, l-arginine, and l-citrulline in the monkey CSF and serum collected on Days 0, 7, 14, and 21 after SAH; 50 μM methionine sulfone was added to each sample as an internal standard and 80 μl of both CSF and serum samples were derivatized with phenylisothiocyanate. The chromatography conditions and quantification were similar to those described by the manufacturer of the PICO-TAG system for analysis of free amino acids (Waters Associates, Milford, MA). After separation on the PICO Tag Column at 46°C for a free amino acid analysis (3.9 × 30 cm column), which was performed using a gradient elution profile (Eliuent A: sodium acetate trihydrate, triethylamine, and acetonitrile [pH 6.5]; Eliuent B: acetonitrile and methanol) with a flow rate of 1.1 ml/minute, the free amino acids were measured with the aid of an ultraviolet light detector (Waters Model 440 absorbance detector) at a wavelength of 254 nm. Eluents A and B were prepared in the manner described by Bidlingmeyer, et al. Starting with 100% Buffer A, the gradient changed over 53.5 minutes to 0% Buffer A/100% Buffer B. Both buffers were constantly degassed inside the autosampler. The detection limit was 10 nM. For data acquisition and processing, Millenium chromatography manager software (Waters Associates) was used. The stability of the ADMA was tested in repeated measurements of samples stored at −80°C for 1 year and no significant degradation was detected (p = 0.34). This is comparable to results presented by Teerink, et al., who showed that the intraassay coefficient of values was 2% for ADMA over a 1-year period in pooled plasma samples stored at −80°C.

Detection of NO Metabolite Levels

The concentrations of both NO− and NO3 in CSF and serum were determined by measuring chemiluminescence with the aid of an NO analyzer (model 280; Seivers Instruments, Inc., Boulder, CO). Samples were thawed on ice and aliquots were injected into an anion-purged reaction vessel containing solutions of either NaI/glacial acetic acid or VCl3/HCl (1 mM at 90°C) to reduce either NO− or NO3, respectively. Nitric oxide was subsequently drawn by vacuum into the detector and reacted with O2. Light formation was quantified and integrated with a photomultiplier tube–computer system. Values were based on signals generated using NaN3 and NaN3 standards.

Western Blot Analysis

Proteins were isolated from the right and left MCAs of control monkeys (five animals) and monkeys with (four animals) and without (two animals) vasospasm on Day 7 post-SAH by using a lysis buffer containing 50 mM Tris (pH 7.5), 0.1% sodium dodecyl sulfate, 1% NP40 (Igepal), 1 mM ethylenediaminetetraacetic acid, 600 mM NaVO4, 50 mM NaF, complete protease inhibitor, 1 mM dithiotreitol, and 1 mM phenylmethylsulfonyl fluoride. The protein content of the lysates was determined by performing a protein assay (Bio-Rad, Hercules, CA), and 20-μg protein samples were subjected to polyacrylamide gel electrophoresis on 8 to 16% gradient Tris–glycine acrylamide gels (Novex–Invitrogen, Carlsbad, CA). Following their transfer to Immunolon P polyvinylidene fluoride membrane (Millipore, Billerica, MA), the samples were probed with rabbit polyclonal ENOS, mouse PRMT1 antibody, and goat polyclonal DDAH2 antibody (1:2000; Novus Biologicals, Littleton, CO). Bands were made visible by applying horseradish-peroxidase–conjugated secondary antibodies (1:2000; Sigma Chemical Co., St. Louis, MO) and a chemiluminescent substrate (Pierce, Rockford, IL). A densitometric analysis was performed.

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Adapted from the original document, here are the key findings and results:

**Results**

**Arteriographic Vasospasm**

Vasospasm developed in the right MCA in 11 of the 16 animals with SAH. The degree of vasospasm in all animals with SAH peaked on Day 7 (45.2 ± 19% reduction from the baseline value on Day 0; p < 0.001), significantly decreased on Day 14 (23.6 ± 5%; four animals; p < 0.01 compared with Day 7), and resolved on Day 21 (2.1 ± 4.7%; five animals; p < 0.001 compared with Day 7 and p < 0.01 compared with Day 14).

**High-Performance Liquid Chromatography**

In CSF and serum, the levels of free ADMA, L-arginine, and L-citrulline were clearly detectable with good separation of the single peaks at retention times of approximately 35, 21, and 23 minutes, respectively.

**Levels of ADMA**

In the control group (six monkeys) the ADMA levels in CSF were 2.7 ± 1.9 μM, which were not different from the ADMA levels of animals in the SAH group (16 monkeys) on Day 0 (2.8 ± 1.9 μM; p = 0.99). On Day 7, the ADMA levels in CSF significantly increased in the SAH group (5.3 ± 2.8 μM; p < 0.05; Fig. 1 upper) and gradually decreased back to control levels between Days 14 and 21 (from 4.5 ± 0.9 μM to 2.7 ± 0.8 μM, respectively, in five animals). Within the SAH group, however, only animals that experienced vasospasm on Day 7 had increased CSF levels of ADMA (Fig. 1 lower). The CSF levels of ADMA in monkeys with vasospasm after SAH (11 animals) significantly increased from Day 0 to Day 7 (from 3 ± 2.4 μM to 7.2 ± 1.9 μM; p < 0.01) and decreased between Days 14 and 21 (from 5.1 ± 0.5 μM to 2.3 ± 0.5 μM in three animals; p < 0.05 compared with Day 7). The levels of ADMA in animals with SAH in which vasospasm did not develop (five animals) remained unchanged after the hemorrhage (Days 0 to 21: mean 2.6 ± 0.8 μM; median: 2.5 μM; range 1.6–3.9 μM; p = 0.17) and were significantly lower compared with ADMA levels in animals with vasospasm on Day 7 (p < 0.01) and Day 14 (p < 0.04; Fig. 1 lower). The ADMA levels in CSF were strongly correlated with the degree of vasospasm (cc = 0.7, p = 0.0001; 95% CI 0.43–0.83).

Asymmetric dimethyl-L-arginine levels in serum of the control group (5.8 ± 2.9 μM) were comparable to those detected in animals in the SAH group on all days after SAH (Days 0–21: mean 7.3 ± 5.8 μM, median 4.8 μM, and range 1.1–24.8 μM; p = 0.43). Levels of ADMA serum in animals with and without vasospasm remained essentially unchanged and did not demonstrate any significant difference on Day 7 (7.5 ± 5.4 μM and 12.5 ± 6.7 μM, respectively; p = 0.27, respectively). Furthermore, ADMA levels in serum were not correlated with ADMA levels in CSF (cc = −0.1; p = 0.66; 95% CI −0.5 to 0.3) or with the degree of vasospasm (cc = −0.28; p = 0.24; 95% CI −0.65 to 0.2).
Asymmetric dimethyl-l-arginine in vasospasm after SAH

Levels of l-arginine in CSF did not differ between the control group (38.8 ± 15.2 μM in six animals) and the SAH group at any time point (Days 0–21; mean 40.5 μM ± 24.4 μM; median 35.5 μM; range 7.4–94 μM; p = 0.73) and remained unchanged in animals with and without vasospasm (p = 0.45). There were also no differences in serum levels of l-arginine between control animals and SAH animals with and without vasospasm on Days 0 to 21 (mean 57.3 ± 13.6 μM in control animals compared with mean 55.4 ± 13.6 μM, median 56.1 μM, and range 26.8–86.6 μM; p = 0.09). The l-arginine levels in CSF and those in serum were not correlated with ADMA levels in either CSF or serum or with the degree of vasospasm.

Levels of l-Citrulline

Levels of l-citrulline in CSF in the control group (5.5 ± 1.2 μM) were similar to levels in the SAH group on Days 0 to 21 (mean 7.3 ± 5.3 μM, median 5.4, range 1.9–24.6 μM; p > 0.05). Although the CSF levels of l-citrulline in animals with vasospasm were not significantly different from those in animals without vasospasm, in the other group the CSF levels of l-citrulline decreased significantly from Day 0 to Day 7 and Day 21 (from 9.1 ± 4.4 μM of Day 0 to 3.6 ± 1.1 μM on Day 7 and 3.8 ± 1.4 μM on Day 21; p < 0.05). The CSF levels of l-citrulline were negatively correlated with their CSF levels of ADMA (cc = −0.51; p = 0.003; 95% CI −0.73 to −0.2) and with the degree of vasospasm (cc = −0.36; p = 0.04; 95% CI −0.62 to −0.02).

In serum the l-citrulline levels in the control group (43.5 ± 16.4 μM) were similar to those in the SAH group (Days 0–21; mean 44.1 ± 13.4 μM, median 40.4 μM; range 26.2–68.1 μM; p = 0.89) and remained unchanged in animals with and without vasospasm after SAH. No correlation was found between the serum levels of l-citrulline and ADMA or the degree of vasospasm.

Levels of NO Metabolites

In four animals in the SAH group the NO2− and NO3− levels were measured in CSF and serum on Days 0 and 7. These animals developed moderate to severe vasospasm post-SAH; the right MCA area decreased by 37 ± 18%. The CSF levels of NO2− were 2.3 ± 1.3 μM on Day 0 and decreased to 1.3 ± 0.8 μM on Day 7 (p < 0.05), and the CSF levels of NO3− were 12.7 ± 6.6 μM on Day 0 and decreased to 3.7 ± 1.7 μM on Day 7 (p < 0.04). Serum NO2− levels (Day 0, 1.8 ± 1.3 μM; Day 7, 1.7 ± 1.4 μM; p = 0.87) and NO3− levels (Day 0, 10.7 ± 1.5 μM; Day 7, 12.7 ± 1.5 μM; p = 0.37) remained unchanged.

Expression of eNOS, PRMT1, and DDAH2

No differences between the right and the left MCA were observed in the protein expression of eNOS (42 ± 20% in the right and 54 ± 12% in the left MCA; p = 0.2) and PRMT1 (76 ± 24% and 91 ± 12%, respectively; p = 0.3) in control animals (five animals) and in animals with vasospasm 7 days after SAH (eNOS: 35 ± 4% in the right and 32 ± 10% in the left MCA; p = 0.5; and PRMT1: 72 ± 33% and 82 ± 19%, respectively; p = 0.6; four animals) and without vasospasm (eNOS: 42 ± 9% in the

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**FIG. 2.** Upper Left: Bar graph depicting mean values of DDAH2 expression ± SD (bars) in the right (R; black bar) and left (L; gray bar) MCAs of control animals and SAH animals on Day 7. n.s. = no significant difference was detected between the right and left MCA in control animals and the left MCA of animals on Day 7 after SAH. # = There was a significant difference in expression in DDAH2 between the right and left MCA in the SAH group (p < 0.05). Upper Right: Bar graph demonstrating mean values of DDAH2 expression ± SD (bars) in the right and left MCAs in animals on Day 7 after SAH. Black bars represent DDAH2 expression in the right and left MCAs of animals with vasospasm; striped bars = animals without vasospasm. *A significant difference was detected between the right and left MCA on Day 7, which was produced by decreased DDAH2 expression in the right MCA of animals with vasospasm (p < 0.03). Lower: Western blot depicting expression of DDAH2 in the right and left MCAs of two animals (1 and 2) with vasospasm (VS) and two animals without vasospasm (3 and 4; nVS) on Day 7 after SAH. Quantification of the band intensity indicated a fourfold decrease in DDAH2 in the right spastic MCA.
right and 30 ± 3% in the left MCA; p = 0.4 and PRMT1: 100 ± 0% and 94 ± 6%, respectively; p = 0.4; two animals). Mainly found in eNOS-expressing tissues, DDAH2 was detected equally in the right and left MCA of control animals (35 ± 16% and 41 ± 15%, respectively; p = 0.1) and in animals without vasospasm after SAH (33 ± 2% and 36 ± 3%, respectively; p = 0.1; Fig. 2 upper left). Nevertheless, DDAH2 expression was significantly attenuated in the right vasospastic MCA (13 ± 18%; p < 0.03) compared with the nonvasospastic left MCA (52 ± 12%) in animals with vasospasm on Day 7 after SAH (Fig. 2 upper right and lower). The difference, however, between DDAH2 expression in the right and left MCA was significantly inversely correlated with the degree of vasospasm (cc = 0.68; p = 0.02; 95% CI 0.13–0.9).

Discussion

The main findings of our experiments are the following: 1) in CSF, the levels of ADMA, an endogenous NOS inhibitor, strongly correlate with the degree of vasospasm after SAH; 2) the levels of L-citrulline, the end product of NO and ADMA metabolism, decrease in the CSF of animals with vasospasm; 3) NO− and NO− levels decrease in the CSF of animals with vasospasm; 4) DDAH2 decreases in arteries in spasm after SAH; and 5) eNOS expression remains unchanged in arteries in spasm.

Recent studies indicate that SAH leads to endothelial dysfunction with decreased availability of NO, reducing the endothelium-dependent, NO-mediated relaxation of the cerebral vasculature. Following SAH, cyclic guanosine-3′,5′-monophosphate levels are decreased in arteries in spasm. A decreased neuronal NOS immunoreactivity has been reported after SAH in primates, although there is controversy over whether cerebral and vascular expression of eNOS is altered by SAH. Park, et al. observed a reduction in eNOS protein in rats only 20 minutes after SAH. A 56% reduction in eNOS mRNA in a primate model reported by Hino, et al. was not accompanied by a decrease in eNOS protein in the ipsilateral brain 7 days after SAH. The expression of eNOS protein remains unchanged in spastic canine basilar arteries 7 days after SAH and in mice 3 days post-SA H. Furthermore, the immunoreactivity of eNOS mRNA remains unchanged or increases in a primate model of delayed cerebral vasospasm.

Our findings support the hypothesis that decreased production of NO is a source of endothelial dysfunction after SAH and that this decrease in the availability of NO in the arterial wall contributes to the development of vasospasm. From our data we can infer that such a vasospasm-associated dysfunction of eNOS is evoked by endogenous inhibition of NOS by ADMA. The increased levels of ADMA are associated with a decrease in the expression of DDAH2, the enzyme responsible for inactivation of ADMA by its hydrolysis to L-citrulline and diethylamine.

Cerebral Vasospasm and ADMA

Treatment of cerebral arteries in vitro and in vivo with exogenous ADMA produces cerebral artery constriction comparable to the vasospasm observed after SAH. We detected ADMA in the CSF and serum of both control monkeys and those with SAH. In the CSF the ADMA levels increased, whereas in the serum these levels remained unchanged after SAH. In monkeys with vasospasm the CSF levels of ADMA peaked on Day 7 post-SA H and decreased on Days 14 through 21; this follows the time course of the development and resolution of cerebral vasospasm in primates and humans. The degree of vasospasm seen on arteriograms was strongly correlated with the CSF levels of ADMA. Moreover, in monkeys with vasospasm the ADMA concentration in CSF was consistent with levels that constrict arteries in vivo; in rats 10 µM of exogenously administered ADMA produces vasoconstriction. Also, in vitro ADMA markedly inhibits the vasodilation of animal and human vessels in response to acetylcholine without inhibiting vasodilation in response to sodium nitroprusside; this emphasizes the differences between endothelium- and NO-dependent and endothelium-independent vasodilatation. Recently, we have shown in vivo that cerebral vessels in spasm following SAH lose their ability to dilate in response to an intracarotid injection of acetylcholine; instead of dilating they constrict. These changes in vessel reactivity are similar to the response of endothelium-denuded artery segments to acetylcholine as described initially by Furchgott and Zawadzki in 1980, indicating the dysfunction of eNOS in the intima of arteries in spasm after SAH.

Previously, we also demonstrated that during vasospasm post-SA H primate vessels maintain the ability to dilate when exposed to an exogenous intracarotid infusion of NO or NO donors. Together these findings support the hypothesis that endothelial dysfunction develops after SAH and indicate that the suppression of eNOS activity by ADMA may contribute to the development of delayed cerebral vasospasm after SAH.

Mechanisms of Increased ADMA

Asymmetric dimethyl-L-arginine is synthesized by PRMT1, which posttranslationally methylates protein-bound L-arginine. Cleavage of dimethylarginine-containing proteins by arginase; hydrolase releases free ADMA into the cytoplasm, where it competitively inhibits eNOS, leading to endothelial dysfunction and vascular constriction. Two isoforms of ADMA hydrolases (DDAH) have been identified that can cleave ADMA into dimethylamine and L-citrulline: 17–19 DDAH1, which predominates in tissues that express nNOS, and DDAH2, a 29644-Dalton protein (Swiss Protein Database, Swiss-Prot Accession No. Q95865), which is mainly found in tissues containing eNOS. Therefore, two major mechanisms may hypothetically lead to an elevation in the ADMA level: 1) increased methylation of protein-bound L-arginine by upregulation of PRMT; and 2) decreased hydrolysis of ADMA by DDAH. Boger, et al., reported an upregulation of PRMT mRNA expression in human endothelial cells by native and oxidized low-density lipoproteins and suggested that elevated PRMT expression is responsible for the elevation of ADMA levels in the setting of hypercholesterolemia. Nevertheless, we observed no increase in PRMT expression in arteries in spasm. On the other hand, DDAH is responsible for the hydrolysis of ADMA and pharmacological inhibition of
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DDAH increases ADMA levels in endothelial cells, leading to blockage of NO-mediated relaxation of blood vessels. Furthermore, a decline in DDAH activity occurs in pathological conditions associated with endothelial dysfunction such as hypercholesterolemia. The expression of DDAH2 was equally detectable in the right and left MCAs of control animals, but was attenuated in the right MCA of animals with vasospasm on Day 7 post-SAH. Additionally, in the CSF the levels of L-citrulline, a product of both ADMA hydrolysis and NO production, decreased in animals with vasospasm and were negatively correlated with the amount of ADMA in the CSF and the degree of vasospasm. Levels of L-arginine, the substrate for NO production and an ADMA competitor for eNOS, remained unchanged in the CSF during this experiment. Altogether these results are consistent with the decreased endothelial DDAH2 activity in the arteries after SAH, which led to increased ADMA levels in the CSF of monkeys with vasospasm and produced endothelial dysfunction through inhibition of eNOS. The actual mechanism by which DDAH2 is inhibited after SAH is unknown.

Earlier studies in this primate model revealed increased CBF after infusion of L-arginine. Vasodilation of preconstricted arteries was also described after exogenous intracisternal L-arginine administration in rabbits as well as in a double-hemorrhage canine model. Both observations indicate that L-arginine may eventually overcome the competitive inhibition of eNOS by ADMA in the presence of vasospasm and further support our hypothesis of ADMA’s involvement in the development of vasospasm after SAH.

Conclusions

In a primate model of SAH the levels of ADMA in the CSF significantly increased concurrently with vasospasm and decreased with vasospasm resolution. The levels of ADMA were strongly correlated with the degree of arteriographic vasospasm. Expression of DDAH2, the enzyme that metabolizes ADMA to L-citrulline and dimethylamine, decreased in vasospastic arteries. These results together with the finding of decreased concentrations of NO2− and NO3− and L-citrulline in the CSF during vasospasm strongly indicate that endogenous inhibition of eNOS by ADMA may initiate and/or sustain delayed cerebral vasospasm after SAH. The involvement of ADMA in the etiology of SAH may open the search for new therapeutic modalities for cerebral vasospasm after SAH.

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