Reversal of delayed vasospasm by an inhibitor of the synthesis of 20-HETE

Kazuhiko Takeuchi,1 Marija Renic,1 Quinn C. Bohman,1 David R. Harder,2 Noriyuki Miyata,3 and Richard J. Roman1

1Department of Physiology and 2Cardiovascular Research Center, Medical College of Wisconsin, Milwaukee, Wisconsin; and 3Medicinal Research Laboratories, Taisho Pharmaceutical, Saitama, Japan

Submitted 27 May 2005; accepted in final form 7 June 2005.

Takeuchi, Kazuhiko, Marija Renic, Quinn C. Bohman, David R. Harder, Noriyuki Miyata, and Richard J. Roman. Reversal of delayed vasospasm by an inhibitor of the synthesis of 20-HETE. Am J Physiol Heart Circ Physiol 289: H2203–H2211, 2005.—This study characterized the time course of changes in cerebral blood flow (CBF) and vascular diameter in a dual-hemorrhage model of subarachnoid hemorrhage (SAH) in rats and examined whether acute blockade of the synthesis of 20-hydroxyeicosatetraenoic acid (20-HETE) with N-(3-chloro-4-morpholin-4-yl)phenyl-N'-hydroxymido formamide (TS-011) can reverse delayed vasospasm in this model. Rats received an intracisternal injection of blood (0.4 ml) on day 0 and a second injection 2 days later. CBF was sequentionally measured using laser-Doppler flowmetry, and the diameters of the cerebral arteries were determined after filling the cerebral vasculature with a casting compound. CBF fell to 67% of control after the first intracisternal injection of blood but returned to a value near control 24 h later. CBF again fell to 63% of control after a second intracisternal injection of blood and remained 30% below control for 5 days. The fall in CBF after the second intracisternal injection of blood was associated with a sustained 30% reduction in the diameters of the middle cerebral, posterior communicating, and basilar arteries. Acute blockade of the synthesis of 20-HETE with TS-011 (0.1 mg/kg iv), 5 days after the second SAH, increased the diameters of the cerebral arteries, and CBF returned to control. These results indicate that the rats develop delayed vasospasm after induction of the dual-hemorrhage model of SAH and that blockade of the synthesis of 20-HETE fully reverses cerebral vasospasm in this model. They also implicate 20-HETE in the development and maintenance of delayed cerebral vasospasm.

The mechanisms of delayed vasospasm remain to be established. Previous studies have indicated that delayed vasospasm is associated with activation of protein kinase C (PKC) (22, 23, 55) and Rho/Rho-kinase (23, 55), diminished K+ channel activity (1, 46), and depolarization of vascular smooth muscle cells (15). The responses of cerebral arteries to endothelin, serotonin, and other vasoconstrictors are elevated, and there is a diminished response to nitric oxide (NO) (14, 15, 47, 48). The levels of endothelin (6, 44), thromboxane (7, 38), ATP (26, 58), isoprostane (43), glutamate (3), platelet-activating factor (PAF) (16), and serotonin (5-HT) (5, 42) in cerebrospinal fluid (CSF) increase after SAH, and the development of cerebral vasospasm can be attenuated by blocking the synthesis of endothelin or by using Ras (57), Rho/Rho-kinase (23, 37), mitogen-activated protein kinase (MAPK) (20), and PKC (23, 37) inhibitors.

Recent studies have drawn attention to the role of 20-hydroxyeicosatetraenoic acid (20-HETE) in the development of cerebral vasospasm. 20-HETE is a potent vasoconstrictor that is produced by the metabolism of arachidonic acid (AA) by cytochrome P-4504A (CYP) enzymes in cerebral arteries (11, 12, 25). The vasoconstrictor response to 20-HETE mimics the changes in cerebral vascular tone associated with cerebral vasospasm. 20-HETE activates PKC (24, 36), Ras, tyrosine kinase, MAPK, and Rho/Rho-kinase pathways (32–35, 40, 50). It promotes calcium entry by depolarizing (25) cerebral arteries secondary to blockade of the large-conductance Ca2+-activated (KCa) channel (24, 49). 20-HETE also increases Ca2+ influx by activating L-type Ca2+ channels in the cerebral vasculature (12). The concentration of 20-HETE in CSF increases markedly after SAH, and inhibitors of the synthesis (5, 18) or actions (59) of 20-HETE prevent the acute fall in CBF after SAH in rats. However, the role of 20-HETE in the development of delayed vasospasm remains to be explored.

Delayed vasospasm has typically been studied in dogs or monkeys using a dual-hemorrhage model of SAH (29). These models faithfully reproduce the time course of the changes in the diameter of cerebral arteries after SAH in humans; however, CBF has not been well characterized, and dogs and monkeys do not develop neurological deficits (29). The single-injection model of SAH has been widely used for the study of acute vasospasm in rats. However, because CBF returns to control within 24 h, many investigators have concluded that rats are not a suitable model system for the study of delayed vasospasm (9, 29). However, this perception is changing because more recent studies have suggested that a sustained reduction in the diameter of cerebral arteries can be elicited
after second intracisternal injection of blood in rats (28, 51, 56). Thus the purpose of the present study is to characterize the time course of changes in CBF and the diameter of cerebral arteries using a dual-hemorrhage model of SAH in rats to confirm that they develop delayed vasospasm and to determine whether acute blockade of the synthesis of 20-HETE with a selective inhibitor of the synthesis of 20-HETE, N-(3-chloro-4-morpholin-4-yl)phenyl-N'-hydroxyimido formamide (TS-011) (31), reverses the delayed vasospasm in this model.

**METHODS**

Experiments were performed on 83 male Sprague-Dawley rats weighing 300–400 g. The rats were housed in an American Association for Accreditation of Laboratory Animal Care-accredited animal care facility at the Medical College of Wisconsin, and they had free access to food and water throughout the study. All experimental procedures were approved by the Animal Care and Use Committee of the Medical College of Wisconsin and conformed to the Guide for the Care and Use of Laboratory Animals of the American Physiological Society.

**Surgical preparation for chronic monitoring of CBF.** The rats were anesthetized with 2% isoflurane (Abbott, Abbott Park, IL) and placed in a stereotaxic apparatus (Stoelting, Wood Dale, IL). A 3 × 3 mm area of the left and right parietal bones overlying the irradiation area of the middle cerebral artery (MCA), 2 mm posterior and 6 mm lateral to the bregma, were thinned with a hand-held drill until the superficial pial vessels were visible. A 10-mm length of polyethylene tubing with the ends heat-flared was affixed over the cranial windows with Vetbond adhesive (3M, Minneapolis, MN) to serve as positioning guide for the laser-Doppler flowmeter probes. The guides were further fixed to the skull with dental acrylic cement, and the scalp incisions were closed around the probe guides with 4-0 silk suture. After surgery, the rats received enrofloxacin (10 mg/kg im, Bayer, Pittsburgh, PA) and buprenorphine (0.1 mg/kg sc, Reckitt Benckiser, Richmond, VA) to prevent infection and relieve pain. The rats were given 5 days to recover from surgery before CBF was measured. This recovery period was necessary because baseline CBF flow was elevated for several days after chronic cranial window surgery because of local inflammation.

**Protocol 1: Characterization of the time course of changes in CBF in the dual-hemorrhage model of SAH in rats.** These experiments were performed using rats prepared for chronic monitoring of CBF as described above. After a 5-day recovery period, rats were anesthetized with isoflurane (2.0%). A microrenathane catheter was chronically fixed to the skull with dental acrylic cement, and the scalp incisions were covered with Vetbond adhesive (3M, Minneapolis, MN) to serve as positioning guide for the laser-Doppler flowmeter probes. The guides were further fixed to the skull with dental acrylic cement, and the scalp incisions were closed around the probe guides with 4-0 silk suture. After surgery, the rats received enrofloxacin (10 mg/kg im, Bayer, Pittsburgh, PA) and buprenorphine (0.1 mg/kg sc, Reckitt Benckiser, Richmond, VA) to prevent infection and relieve pain. The rats were given 5 days to recover from surgery before CBF was measured. This recovery period was necessary because baseline CBF flow was elevated for several days after chronic cranial window surgery because of local inflammation.

**Protocol 2: Measurement of cerebral vascular diameters at various times after the dual-hemorrhage model of SAH in rats.** These experiments were performed in five groups of rats surgically prepared for induction of the dual-hemorrhage model of SAH. At various times after the induction of SAH, the cerebral circulation was perfusion-fixed and filled with a silicone rubber compound for measurement of vascular diameters. In group 1 (control; n = 6), the cerebral circulation was filled before the induction of SAH on day 0. In group 2 (acute SAH; n = 6), the cerebral circulation was filled 30 min after the induction of acute SAH on day 0. In group 3 (delayed vasospasm-day 3; n = 6), the cerebral circulation was filled 1 day after the second intracisternal injection of blood. In group 4 (delayed vasospasm-day 7; n = 6), the cerebral circulation was filled 5 days after the second intracisternal injection of blood. In group 5 (vehicle control-day 7; n = 6), rats received intracisternal injection of saline on days 0 and 2, and the cerebral circulation was filled 5 days after the second intracisternal injection.

At the appropriate times after the induction of SAH, the rats were anesthetized with isoflurane (2.0%), and the right and left carotid arteries were cannulated with polyethylene tubing (PE-50). The cerebral circulation was flushed with 30 ml of a heparinized (20 U/ml) physiological saline solution (PSS) containing (in mM) 119.0 NaCl, 4.7 KCl, 1.6 CaCl2, 1.17 MgSO4, 1.18 NaH2PO4, 12.0 NaHCO3, 0.03 EDTA, 10.0 glucose, and 10.0 HEPES (pH 7.4) that was perfused via the carotid arteries at pressure of 110 mmHg followed by perfusion fixation with another 30 ml of PSS containing 4% of paraformaldehyde. After fixation, the cerebral vessels were filled at 110 mmHg with 12 ml of a silicone rubber casting material (Microfil MV-122, FlowTek, Bounder, CO) that was diluted 1:4 with the diluent supplied by the manufacturer. The casting material was allowed to cure for 4 h. The brain was then removed and placed in cold PSS. The diameter of the filled cerebral arteries was measured using a video system composed of stereomicroscope (Zeiss, Germany), a video camera (COHU-4815, COHU Electronics, Poway, CA), and a video measuring system (VIA-100, Boeckeler Instrument, AZ) to prevent shrinkage. The diameter of the basilar artery (BA) was measured 400 μm above the junction of the vertebral arteries, just below the origin of the anterior inferior cerebellar arteries, and 400 μm below the origin of the posterior cerebral arteries. The diameters of right and left middle cerebral arteries (MCAs) and posterior communicating arteries (PCAs) were measured 400 μm distal to the PCA-MCA bifurcation and 400 μm proximal to the PCA-MCA bifurcation. The minimum diameter measured for each of these arteries was recorded.

**Protocol 3: Effect of TS-011 on the delayed vasospasm in the dual-hemorrhage model of SAH in rats.** These experiments examined the ability of an inhibitor of the synthesis of 20-HETE, TS-011, to reverse delayed cerebral vasospasm in rats. The rats were surgically prepared for chronic measurement of CBF and induction of the dual-hemorrhage model of SAH. The rats were anesthetized with isoflurane (2.0%), and the right and left carotid arteries were cannulated with polyethylene tubing (PE-50). The cerebral circulation was flushed with 30 ml of a heparinized (20 U/ml) physiological saline solution (PSS) containing (in mM) 119.0 NaCl, 4.7 KCl, 1.6 CaCl2, 1.17 MgSO4, 1.18 NaH2PO4, 12.0 NaHCO3, 0.03 EDTA, 10.0 glucose, and 10.0 HEPES (pH 7.4) that was perfused via the carotid arteries at pressure of 110 mmHg followed by perfusion fixation with another 30 ml of PSS containing 4% of paraformaldehyde. After fixation, the cerebral vessels were filled at 110 mmHg with 12 ml of a silicone rubber casting material (Microfil MV-122, FlowTek, Bounder, CO) that was diluted 1:4 with the diluent supplied by the manufacturer. The casting material was allowed to cure for 4 h. The brain was then removed and placed in cold PSS. The diameter of the filled cerebral arteries was measured using a video system composed of stereomicroscope (Zeiss, Germany), a video camera (COHU-4815, COHU Electronics, Poway, CA), and a video measuring system (VIA-100, Boeckeler Instrument, AZ) to prevent shrinkage. The diameter of the basilar artery (BA) was measured 400 μm above the junction of the vertebral arteries, just below the origin of the anterior inferior cerebellar arteries, and 400 μm below the origin of the posterior cerebral arteries. The diameters of right and left middle cerebral arteries (MCAs) and posterior communicating arteries (PCAs) were measured 400 μm distal to the PCA-MCA bifurcation and 400 μm proximal to the PCA-MCA bifurcation. The minimum diameter measured for each of these arteries was recorded.

Experiments were performed to confirm the effectiveness of TS-011 as an inhibitor of the synthesis of 20-HETE in cerebral arteries. MCA and BA were microdissected from the brains of four rats and divided into two samples. These samples were incubated for 1 h at 37°C in 1 ml of a 10 mM potassium phosphate buffer containing 40 μM AA, 1 mM NADPH, and 100 nM TS-011 (n = 3) or vehicle (n = 4). The reactions were stopped by acidification with 1 M formic acid, extracted with ethyl acetate, and dried. The reactions were resuspended in 50% methanol and water, and the products were separated and measured using liquid chromatography/mass spectrometry (LC/MS) on an Agilent 1100 ion-trap mass spectrometer as previously described (8).

Additional experiments were performed to determine whether TS-011 effectively inhibits the synthesis of 20-HETE in the brain after in vivo administration. Rats were anesthetized with isoflurane and given intravenous injection of TS-011 (0.1 mg/kg, n = 4) or vehicle (n = 4). Ninety minutes later, the brains of these animals were collected and homogenized in 2 ml of a 10 mM potassium buffer (pH 7.7) containing (in mM) 250 sucrose, 1 EDTA, and 0.1 phenylmethylsulfonyl fluoride (PMSF). The homogenate was centrifuged at 3,000 g for 15 min, and the supernatant was centrifuged at 11,000 g for 15 min followed by 100,000 g for 1 h. The microsomal pellets were resuspended in 100 mM potassium phosphate buffer containing 40 μM AA, 1 mM NADPH, 10 mM sodium isocitrate, and 0.16 U/ml isocitrate dehydrogenase. The samples were extracted with ethyl acetate, and the production of 20-HETE was determined by LC/MS as previously described (8).

Statistical analysis. Mean values ± SE are presented. CBF was expressed as a percentage of the baseline value measured on day 0, before the intracisternal injection of blood or saline. The significance of changes in CBF within groups was evaluated using an ANOVA for repeated measures followed by Dunnett’s test. The significance of differences in the diameter of cerebral arteries between groups was evaluated using an ANOVA followed by a Duncan’s multiple-range test or an unpaired t-test. P < 0.05 was considered significant.

RESULTS

Protocol 1: Time course of changes in CBF after the dual-hemorrhage model of SAH in rats. The results of these experiments are presented in Fig. 1. CBF was not significantly altered during the experiment in control rats that received intracisternal injections of saline alone. In contrast, CBF fell to 67.2 ± 3.1% of control 30 min after the injection of blood into the cisterna magna. CBF fully recovered to a value near control 24 h later. CBF again fell significantly to 63.8 ± 2.8% of control 30 min after the second intracisternal injection of blood. Thereafter, CBF remained 30% below control when measured 1, 3, or 5 days later.

Fig. 2. Appearance of the cerebral circulation at various times after the dual-hemorrhage model of SAH in rats. A: control. B: acute SAH, 30 min after the first intracisternal injection of blood. C: 1 day after the first intracisternal injection of blood. D: 1 day after the second intracisternal injection of blood.
Protocol 2: Time course of changes in the diameter of cerebral arteries after the dual-hemorrhage model of SAH in rats. The typical appearances of the cerebral circulation after acute SAH, before the second injection of blood on day 2, and 5 days after the second hemorrhage are presented in Figs. 2 and 3. Blood was found to surround the MCA, PCA, and BA, 30 min after intracisternal injection of blood on days 0 or 2 (Fig. 2B), and the diameter of the BA, MCA and PCA exhibited obvious vasospasm (Fig. 2B and 3, C and D). However, the injected blood was nearly cleared 24 h after the first injection of blood (Fig. 2C), and the diameter of these vessels returned toward control. The blood was completely cleared within 1 day after the second intracisternal injection of blood (Fig. 2D), but the diameter of the MCA, PCA, and BA remained constricted for at least 5 days (Fig. 3, E and F). A summary of the vessel diameter data is presented in Fig. 4. Baseline diameters of the MCA, PCA, and BA in control rats averaged 201.0 ± 7.3, 238.1 ± 9.0, and 225.5 ± 8.7 μm, respectively. The diameters of MCA, PCA, and BA fell to 73, 66, and 78% of control 30 min after the first intracisternal injection of blood. The diameters of MCA, PCA and BA measured on days 3 and 7, 1 and 5 days after the second intracisternal injection of blood remained significantly lower than control (Fig. 4).

Protocol 3: Effects of TS-011 on delayed vasospasm in the dual-hemorrhage model of SAH in rats. The effects of inhibition of the synthesis of 20-HETE with TS-011 on CBF in the dual-hemorrhage model are presented in Fig. 5. Administration of TS-011 fully reversed the fall in CBF in rats with delayed vasospasm on day 7. CBF recovered to control within 120 min after a bolus intravenous injection of TS-011 (Fig. 5A). TS-011 had no effect on MAP in these animals (Fig. 5B). We also examined the effects of TS-011 on the diameter of cerebral arteries. A representative appearance of the cerebral circulation after administration of vehicle or TS-011 in rats with delayed vasospasm is presented in Fig. 6A, and a summary of the diameter data is presented in Fig. 6B. The diameter of the MCA, PCA, and BA on day 7 returned to values not different from control after administration of TS-011 to rats subjected to the dual-hemorrhage model of SAH.

are presented in Fig. 7. MCA incubated with AA in vitro produced peaks detected by LC/MS with mass-to-charge ratio (m/z) of 319 that coelute with 20-, 15-, 12-, and 5-HETE and 14,15-epoxyeicosatrienoic acid (EET). Addition of TS-011 (100 nM) to the incubations reduced the formation of 20-HETE by 80% (n = H11005) without affecting the formation of 15-HETE, 12-HETE, or EETs.

We also verified that the dose of TS-011 (0.1 mg/kg iv) used in these studies was sufficient to inhibit the formation of 20-HETE in vivo (Fig. 8). The baseline production of 20-HETE by microsomes prepared from the brains of rats treated with vehicle averaged 6.0 ± 3.1 pmol·min⁻¹·mg protein⁻¹. The production of 20-HETE was significantly reduced by 91% (n = 4) in rats treated with TS-011, whereas the formation of other products of AA, 12-HETE, 11,12- and 14,15-EETs, and 11,12-dihydroxyeicosatetraenoic acid was not significantly altered.

DISCUSSION

The present study characterized the time course of changes in vascular diameter and CBF in a modified dual-hemorrhage model of SAH in rats and examined the contribution of 20-HETE in the development of delayed vasospasm in this model using a selective inhibitor of the synthesis of 20-HETE, TS-011. We found that acute blockade of the synthesis of 20-HETE reversed vasospasm in this model, thereby suggesting that 20-HETE plays a critical role in the development and maintenance of delayed cerebral vasospasm.

Previous studies have documented that there are biphasic changes in the diameter of cerebral arteries in humans after SAH (30, 54). The acute phase lasts several hours. However, over the next 4–7 days, half the patients develop delayed vasospasm that is refractory to treatment with vasodilators or calcium channel blockers (52, 54). Delayed vasospasm has typically been studied angiographically in dogs or monkeys using a dual-hemorrhage model of SAH (29). The diameter of the basilar artery of dogs acutely falls by 30% after the injection of blood into the cisterna magna (21, 52). It then returns to control within 24–48 h, but a delayed vasospasm develops after a second intracisternal injection of blood. While the dual-hemorrhage model of SAH in dogs and monkeys reproduces the time course of the changes in the diameter of cerebral arteries after SAH in humans, CBF has not been well characterized in these models, and dogs and monkeys do not develop neurological deficits (29). In addition, studies performed in these large animal models are very expensive, and this limits the amount of mechanistic work that can be done. The development of a small animal model of delayed vasospasm could offer many advantages. Indeed, many investigators have switched to rats to study the acute fall in CBF after SAH that is associated with a constriction of the MCAs, PCAs, and BA (3, 9, 13, 19). However, CBF and the diameters of the cerebral arteries return to control within 48 h in rats after SAH. This observation has lead most investigators to conclude that...
Rats are not a suitable model for the study of delayed vasospasm (9, 29). However, more recent studies have suggested that the diameter of cerebral arteries is reduced for several days after a second intracisternal injection of blood in rats as is seen in larger animal models (28, 51, 56). These observations led us to characterize the changes in CBF and vascular diameter in rats subjected to a dual-hemorrhage model of SAH to see if rats develop delayed vasospasm.

The present results confirm previous findings that CBF acutely falls after SAH in rats, and this is associated with a reduction of 30–40% in the diameter of MCA, PCA, and BA. We also confirmed that the blood is rapidly cleared from CSF after SAH, and CBF returns to values within 90% of control 24 h later. However, after a second intracisternal injection of blood, there is a 30% fall in CBF along with sustained constriction of MCA, PCA, and BA for 5 days. Most of the rats in the present study also exhibited neurological deficits such as weakness in the front paws. Overall, the present findings indicate that rats subjected to dual-hemorrhage model of SAH develop biphasic changes in CBF and the diameter of the cerebral arteries that follow the same time course (peaks on days 5–7) and are of the same magnitude (30–40%) as that seen in humans or in the dual-hemorrhage models of SAH in dogs or monkeys (29, 30, 52–54, 57).

Fig. 6. Effects of TS-011 on the diameter of cerebral arteries in the dual-hemorrhage model of SAH in rats. A: appearance of the MCA and PCA after bolus intravenous injection of TS-011 or vehicle on day 7, 5 days after the second injection of blood into the cisterna magna of rats. B: summary data on the diameter of MCA and PCA after administration of TS-011 (n = 6) or vehicle (n = 6) on day 7, 5 days after the second injection of blood into the cisterna magna of rats. Control diameter data (control, n = 6) and the data from the SAH-treated group on day 7 (SAH(D7), n = 6) were replotted from the data presented in Fig. 4 to facilitate comparisons. Values are means ± SE. *P < 0.05 vs. corresponding control value. †P < 0.05 vs. corresponding value in vehicle-treated rats.

Fig. 7. Effects of TS-011 on the production of 20-HETE by rat MCA and BA in vitro. Representative liquid chromatographic/mass spectrometric (LC/MS) chromatogram is shown, illustrating the metabolism of arachidonic acid by MCA and BA of rats incubated in the presence and absence of TS-011 (100 nM). TS-011 inhibited the formation of 20-hydroxyeicosatetraenoic acid [20-HETE; mass-to-charge ratio (m/z) of 319] that elutes with a retention time of 16 min, but it did not reduce the synthesis of 15-, 12-, or 5-HETE or 14,15-epoxyeicosatrienoic acid (EET).
Role of 20-HETE in delayed vasospasm in rats. Previous studies have indicated that 20-HETE plays an important role in the development of acute vasospasm after SAH in rats (5, 18). A more recent study has indicated that the levels of 20-HETE in CSF also increase in patients with SAH (39). However, the role of 20-HETE in the development of delayed vasospasm is unknown. To determine if 20-HETE contributes to the increase in cerebral vascular tone in delayed vasospasm, we studied the effects of TS-011, a new and very selective inhibitor of the synthesis of 20-HETE (31), on CBF and cerebral vascular diameter in the dual-hemorrhage model of SAH in rats. We found that TS-011 fully returned CBF to control without affecting MAP, and this was associated with an increase in the diameter of the MCA, PCA, and BA. In further studies, cerebral arteries microdissected from the brains of rats synthesized 20-HETE when incubated with AA in vitro and TS-011 inhibits the formation of this substance. We also found that microsomes prepared from the brains of rats synthesized 20-HETE and that pretreatment of rats in vivo with TS-011 (0.1 mg/kg) selectively inhibited the synthesis of 20-HETE. These observations suggest that upregulation of the synthesis of 20-HETE may contribute to the development of delayed vasospasm. A role for 20-HETE in the development of delayed vasospasm is consistent with previous observations that inhibitors of Ras, Rho/Rho kinase, MAPK, or PKC attenuate the development of delayed cerebral vasospasm (20, 23, 37, 57) because 20-HETE promotes depolarization and contraction of cerebral arteries by activating these same second messenger pathways (2, 24, 50, 59).

The finding that blockade of the synthesis of 20-HETE with TS-011 fully reversed the fall in CBF and reduction in the diameter of the cerebral arteries in rats with delayed vasospasm does not preclude an important role for other mediators in response. Indeed, previous investigators have shown that the levels of endothelin (6, 44), thromboxane (7, 38), ATP (26, 58), isoprostane (43), glutamate (3), PAF (16), and serotonin (5, 42) all increase in CSF after SAH, and the degree of cerebral vasospasm can be attenuated by blocking the synthesis or actions of most of these mediators. Others have reported that elevations in superoxide radicals (19) and a fall in the bioavailability of NO (48) contribute to the development of cerebral vasospasm. The most likely explanation is that many of the vasoactive mediators released by clotting blood likely trigger the development of vasospasm but that many of these pathways converge on a common pathway, leading to elevated production of 20-HETE in cerebral arteries, which potentiates the vasoconstrictor actions of these compounds by depolarizing vascular smooth muscle by blocking the KCa channel. The results of previous studies indicating that 20-HETE contributes to the vasoconstrictor responses to endothelin, ANG II, serotonin, vasopressin, and norepinephrine and inhibition of the synthesis of NO support this possibility (2, 5, 41, 59).

The mechanism responsible for the upregulation of the formation and or actions of 20-HETE after SAH remains to be determined. The expression of inducible nitric oxide synthase is elevated in cerebral arteries after SAH (56). Moreover, heme oxygenase-1, which metabolizes the heme to iron, biliverdin, and carbon monoxide (CO), is also induced in the brain after SAH (27). Both NO and CO avidly bind to heme in CYP enzymes and inhibit the formation of 20-HETE (49). Indeed, induction of the formation of NO and CO may contribute to the rapid recovery of CBF after SAH by activating cGMP-dependent vasodilator pathways and by inhibiting of the formation of the vasoconstrictor 20-HETE. Besides blocking the formation of 20-HETE, NO is known to upregulate the expression of CYP4A enzymes (45). CO might have a similar effect. Up-regulation of the expression CYP4A enzymes and the local formation of 20-HETE in cerebral arteries after SAH might contribute to the development of delayed vasospasm after the clotted blood is cleared from the CSF and the levels of NO and CO return to control.

In summary, the present study indicates that rats subjected to the dual-hemorrhage model of SAH exhibit biphasic changes in CBF and the diameter of cerebral arteries that closely mimic the time course and magnitude of the response previously reported in humans (30, 54) and the dual-hemorrhage dog and monkey models of SAH (29, 52). Acute blockade of the synthesis of 20-HETE fully reversed cerebral vasospasm in this model. These results suggest that 20-HETE plays a critical role in the development and maintenance of delayed cerebral vasospasm.
REFERENCES


