Phosphodiesterase 5 Inhibition Attenuates Cerebral Vasospasm and Improves Functional Recovery after Experimental Subarachnoid Hemorrhage

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Abstract

Background—Cerebral vasospasm is an independent predictor of poor outcome after subarachnoid hemorrhage (SAH). The nitric oxide-cyclic GMP (NO-cGMP) vasodilatory pathway is strongly implicated in its pathophysiology. Preliminary studies suggest that phosphodiesterase 5 (PDE5) – an enzyme that degrades cGMP – may play a role, as the PDE5 inhibitor sildenafil was found to reduce vasospasm after SAH. However, several questions that are critical when considering translational studies remain unanswered.

Objective—To elucidate the mechanism of action of sildenafil against vasospasm, and to assess whether sildenafil attenuates SAH-induced neuronal cell death, improves functional outcome after SAH, or causes significant physiological side effects when administered at therapeutically relevant doses.

Methods—SAH was induced via endovascular perforation in male C57BL6 mice. Beginning two hours later, mice received sildenafil citrate (0.7, 2 or 5mg/kg P.O. BID) or vehicle. Neurological outcome was assessed daily. Vasospasm was determined on post-SAH Day 3. Brain PDE5 expression and activity, cGMP content, neuronal cell death, arterial blood pressure (BP), and intracranial pressure (ICP) were examined.

Results—We found that PDE5 activity (but not expression) is increased after SAH, leading to decreased cGMP levels. Sildenafil attenuates this increase in PDE5 activity and restores cGMP levels after SAH. Post-SAH initiation of sildenafil was found to reduce vasospasm, decrease neuronal cell death, and markedly improve neurological outcome, without causing significant physiological side effects.

Conclusion—Sildenafil–an FDA-approved drug with a proven track record of safety in humans –is a promising new therapy for vasospasm and neurological deficits following SAH.

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INTRODUCTION

Vasospasm-induced delayed cerebral ischemia (DCI) is a major risk factor for poor outcome after SAH, and is the most common cause of secondary neurological injury in this population.[1] Numerous studies suggest that a key contributing factor to vasospasm pathophysiology is downregulation of the NO-cGMP vasodilatory pathway. Evidence for this include reduced NO bioavailability, decreased endothelial nitric oxide synthase, and reduced cGMP (the downstream effector of NO) after SAH.[2] The latter appears to occur (in part) from SAH-induced upregulation of PDE5 – an enzyme that selectively hydrolyzes cGMP to GMP.

Sildenafil citrate (Viagra™) is one of a number of highly selective PDE5 inhibitors that attenuate PDE5-mediated hydrolysis of cGMP leading to increased intracellular cGMP levels, smooth muscle relaxation, and arterial vasodilation.[3] Several recent studies suggest sildenafil and other PDE5 inhibitors likely reduce vasospasm following SAH.[4–8] Though these results are promising, additional investigation is required before considering translational studies in humans. For example, the following have yet to be examined: 1) whether sildenafil reduces neuronal cell death following SAH; 2) whether sildenafil improves neurological outcome following SAH; and 3) whether sildenafil causes systemic hypotension or exacerbates SAH-induced intracranial hypertension when administered at doses that attenuate vasospasm. The molecular pathway by which sildenafil exerts its anti-vasospasm effect has also not been elucidated. We sought to answer these critical questions utilizing an endovascular perforation mouse model of SAH.

MATERIALS AND METHODS

Experimental SAH

All experimental protocols were approved by the Animal Studies Committee at Washington University in St Louis. Three to four month old male C57BL/6J mice (Jackson Laboratories; Bar Harbor, Maine) were used. Endovascular perforation SAH and sham surgery were performed as described.[9] Briefly, mice were anesthetized with Isoflurane (4% induction, 1.5% maintenance). A blunted 5-0 monofilament nylon suture was introduced into the ECA, and advanced through the internal carotid artery (ICA) until the anterior cerebral artery (ACA)–middle cerebral artery (MCA) bifurcation was encountered. For SAH surgery, the suture was advanced 3 mm further to perforate the ACA leading to SAH. For sham surgery, the suture was not advanced. The suture was then removed, the ECA ligated, and the skin incision closed. After recovery, mice were returned to their cages and allowed free access to water and rodent chow. To prevent dehydration post-operatively, saline containing 10% dextrose (0.5 ml) was intraperitoneally administered twice a day for 3 days.

Drug Administration

Sildenafil citrate (Viagra™, Pfizer Inc., New York, NY) was powdered and suspended in saline. Vehicle (saline) or sildenafil (0.7, 2 or 5 mg/kg) was administered by oral gavage twice daily starting 2h after surgery. Low dose was based on the effective dose for erectile dysfunction in humans: 0.7 mg/kg; higher doses were chosen because the elimination half-

life in mice (1.3 h) is less than humans (3.7 h).[10] The time point for initiating sildenafil was based on cerebral ischemia studies.[11]

Physiological Data

To examine BP, naive mice were anesthetized with isoflurane and a polyethylene catheter (PE10, Clay Adams) was inserted into the common carotid artery. Thirty minutes later, BP in awake mice was recorded before and after sildenafil (2 mg/kg) or vehicle administration. To examine ICP, a Mikro-tip pressure catheter (AD Instruments) was placed through a 1-mm right parietal burr hole; ICP was monitored for 10 minutes beginning 3 hours after SAH.

Behavioral Tests

Neurobehavioral outcome was examined daily using Neuroscore and Rotarod tests as described.[9] Briefly, neurological function was graded based on a motor score (0–12) that evaluated spontaneous activity, symmetry of limb movements, climbing, balance and coordination; and a sensory score (4–12) that evaluated body proprioception and vibrissae, visual, and tactile responses. Balance and coordination were assessed by performance on Rotarod (Rotamex-5, Columbus Instruments, Columbus, OH).[9] The latency on three trials of 180 seconds each was averaged daily on Days 0–3. Mice were pre-trained on the Rotarod one day prior to surgery. All assessments were performed by investigators who were blinded to the surgical procedure and treatment group. Functional outcome was assessed in the same groups of mice that were used for vasospasm assessment.

Vasospasm

MCA vasospasm was assessed by cerebrovascular casting method as described with modification.[9, 12] Briefly, under isoflurane anesthesia, mice were transcardially perfused at a pressure of 75±5 mmHg with heparinized phosphate-buffered saline (PBS), followed by 10% formalin, and then casting medium containing 3% gelatin and 25% India ink. Mice were refrigerated overnight to allow gelatin solidification. Brains were then removed and blood vessels imaged using a CCD camera (CoolSNAP ES, Thotometrics, Tucson, AZ). Spasm of the ipsilateral proximal MCA was assessed by recording the narrowest diameter in the first 1000 μm of the MCA. To assess the time course of vasospasm in our model, mice were initially sacrificed on Days 2–4 after SAH. Since Day 3 was found to be the period of maximum vasospasm (see below), mice were sacrificed on Day 3 for subsequent efficacy studies with sildenafil. Vasospasm assessment was performed by investigators who were blinded to the treatment group.

PDE5 Activity

PDE5 activity was determined by measuring cGMP-hydrolyzing activity as described.[7] Ten microliters of brain lysates were added to 90 μL of reaction buffer (20 mM HEPES, pH 7.4, 5 mM $MgCl_2$, 1 mM DTT, 42 mM KCl, 10% sucrose and protease inhibitor cocktail) containing 1 μM cGMP with or without the PDE5 inhibitor, zaprinast (10μM).[13] After incubating at 37°C for 30 min, the reaction was stopped by heating at 95°C for 5 min. The remaining cGMP content was determined by cGMP ELISA assay. Total PDE and PDE5

activities were calculated as pmol/min/mg protein. Investigators were blinded to the surgical procedure (Sham or SAH) and treatment group (Vehicle or Sildenafil).

Western Blot

Western blots were performed on cerebral cortices ipsilateral to endovascular perforation as described.[9, 14] The following primary antibodies were used: rabbit polyclonal anti-PDE5 1:500 (Santa Cruz Biotechnology Inc) and mouse monoclonal anti alpha-Tubulin 1:20000 (Sigma). Blots were subsequently incubated with anti-rabbit or anti-mouse horseradish peroxidase-conjugated IgG (Biorad, Hercules, CA) and visualized using an enhanced chemiluminescence kit (Biorad, Hercules, CA). Alpha-tubulin was used as the internal control. Investigators were blinded to the surgical procedure (Sham or SAH) and treatment group (Vehicle or Sildenafil).

cGMP ELISA

Ipsilateral brain tissue was lysed in a buffer containing 10 mM HEPES, pH, 7.4, 1% Triton X-100, 5 mM $MgCl_2$, 1 mM DTT, and protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO).[14] Tissue lysates were centrifuged at $10,000 \times g$ at 4°C for 10 min and the supernatants (10 μl) subjected to cGMP ELISA assay (R&D Systems, Inc., Minneapolis, MN) according to the manufacturer's protocol. Investigators were blinded to the surgical procedure (Sham or SAH) and treatment group (Vehicle or Sildenafil).

Immunohistochemistry

Free floating coronal brain sections (40 μm) were subjected to immunostaining with anti-cGMP antibody (1:500; Chemicon, Millipore, Billerica, MA)using a Vectastain Elite ABC-peroxidase kit (Vector Laboratories) and Tyramide signal amplification kit (Invitrogen), as described.[14] Smooth muscle cells were labelled with Phalloidin-Alexa 568 (Invitrogen).

Terminal deoxynucleotidyl transferase-dUTP nick end labeling (TUNEL)

Equally-spaced brain sections (4 sections per mouse) were used. DNA fragmentation was detected using a TUNEL apoptosis detection kit (Invitrogen) as described.[15] TUNEL-positive cells in the ipsilateral parietal cortex were quantified (4 fields per section). Investigators were blinded to the treatment group (Vehicle or Sildenafil).

Statistical analysis

Data are presented as the mean ± SEM. All data except functional outcome were analyzed by ANOVA followed by Newman-Keuls multiple comparison method. For functional outcome, Neuroscore (after rank transformation) and Rotarod data were analyzed using Repeated Measures ANOVA and post-hoc Fisher's Least Significant Difference method;A $p < 0.05$ was considered as statistically significant for all analyses.

RESULTS

SAH causes vasospasm

Endovascular suture-perforation consistently produced SAH around the left ICA, MCA, and ACA and the extended subarachnoid space with minimal post-operative mortality (<10%) (Fig. 1A,B). Vasospasm of the MCA was consistently noted, with peak vasospasm occurring on post-SAH Day 3 (n=10, 5, 6 and 5 for Sham, SAH:Day 2, SAH:Day 3 and SAH:Day 4 groups respectively) (Fig. 1C,D). Histology confirmed marked narrowing of the arterial lumen as well as intimal corrugation of the ipsilateral cerebral arteries (Fig. 1E).

Sildenafil inhibits SAH-induced increase in PDE5 activity

An increase in zaprinast-sensitive PDE5 activity was noted in the ipsilateral hemisphere 3 d post-SAH as compared with sham-operated mice [SAH:vehicle (n=7): 538.4±118.5 vs. Sham:vehicle (n=3): 250.0±84.6 fmol/min/mg protein, P=0.07] (Fig. 2A). Sildenafil strongly attenuates this increase in PDE5 activity [SAH:sildenafil (n=5): 81.5±110.5 vs. SAH:vehicle (n=7): 538.4±118.5 fmol/min/mg protein, P<0.05]. Total cGMP-hydrolyzing activity (by all PDEs) was unaltered in SAH mice ± sildenafil as compared with sham-operated mice (P>0.05) (Fig 2B). These data indicate that PDE5 is the predominant PDE isoform that is activated following SAH, and that sildenafil attenuates this activation. Western blot analysis showed no significant difference between groups in PDE5 expression (Fig 2C), suggesting that SAH-induced PDE5 upregulation is likely due to post-translational modifications.

Sildenafil inhibits SAH-induced cGMP breakdown

Immunohistochemical labeling with anti-cGMP antibody and confocal microscopy revealed a marked decrease in cGMP immunoreactivity in cerebral arteries and neurons following SAH (n=6) vs. sham-operated mice (n=4) (Fig 2D); sildenafil (2mg/kg) (n=6) restores cGMP levels after SAH (Fig. 2D). These findings were confirmed by cGMP ELISA assay (Fig. 2E). cGMP content in the ipsilateral cortex was decreased 3 d post-SAH vs. sham-operated mice [SAH:vehicle (n=7): 150±27 vs. Sham:vehicle (n=3): 456±134 fmol/mg protein, P <0.05]; sildenafil attenuates this SAH-induced drop in cGMP [SAH:sildenafil (n=5): 389±128 vs. SAH:vehicle (n=7): 150±27 fmol/mg protein, P<0.05].

Sildenafil attenuates vasospasm after SAH

Sildenafil (2 mg/kg) did not alter MCA caliber in sham-operated mice [Sham:sildenafil (2mg/kg) (n=20): 103.4±8.1 vs. Sham:vehicle (n=15): 103.9±5.1, p>0.05). Importantly, all three doses of sildenafil reduced MCA vasospasm in SAH-operated mice [SAH:vehicle (n=19): 70.1±5.3 vs. SAH:sildenafil (0.7mg/kg) (n=14): 92.8±4.1, SAH:sildenafil (2mg/kg) (n=20): 92.9±6.6 μm, and SAH:sildenafil (5mg/kg) (n=13): 95.3±6.9; P<0.05 for all three groups vs. SAH:vehicle] (Fig. 3 A,B). No significant differences in mortality were noted between groups (data not shown).

Sildenafil improves functional outcome after SAH

Sildenafil reduces SAH-induced neurological deficits in a dose dependent manner (*P*<0.05) as assessed by Neuroscore (Fig. 4A) and Rotarod test (Fig. 4B). Sildenafil (2mg/kg) has no effect on the performance of sham-operated mice.

Sildenafil attenuates neuronal cell death after SAH

There was a significant increase in the number of TUNEL positive neurons in the ipsilateral parietal cortex after SAH [Sham:vehicle (n=4): 2.0±0.9 vs. SAH:vehicle (n=3): 35±6.3 cells/field (field = 83,083µm^2), p<0.05). Sildenafil (2mg/kg) results in a 55% reduction in TUNEL positive neurons following SAH (SAH:vehicle (n=3): 35±6.3 cells/field (field = 83,083µm^2) vs. SAH:sildenafil (2mg/kg) (n=4): 15.1±2.8 cells/field, p<0.05) (Fig 5).

Sildenafil has no effect on BP and ICP

In normal conscious mice (n=3/group), sildenafil (2 mg/kg) does not affect mean arterial pressure for up to 3 hours post-injection (Fig 6A). In mice subjected to SAH, sildenafil (2 mg/kg) does not exacerbate SAH-induced intracranial hypertension [SAH:vehicle (n=4): 21.1±2.3 vs. SAH:sildenafil (n=4): 16.6±2.3 mmHg, *P*>0.05) (Fig 6B).

DISCUSSION

Following SAH, several molecular events occur that alter the balance of the NO-cGMP vasodilatory pathway such that it has become a prime target for anti-vasospasm therapy. First, SAH causes decreased NO bioavailability via a multitude of pathways.[2] Based on this finding, many therapeutic strategies for increasing NO availability have been pursued; however, mixed results have thus far been encountered.[16] This is likely due to the short half life[17] and unpredictable distribution of NO,[2] downstream alteration in PDE5 activity that could impede NO-induced vasodilation,[7,18] and side effects of NO-directed therapy including systemic hypotension,[19] vascular steal,[20] and increased ICP.[19]

Second, SAH causes impaired vascular responsiveness to NO by decreasing cGMP availability. This has been attributed to increased PDE5 expression[7] and/or activity,[7, 18] which has made PDE5 inhibition a novel and attractive anti-vasospasm strategy. Studies to date show the following: 1) acute administration of sildenafil partially relaxes basilar artery vasospasm in dogs[7]; 2) pre-SAH initiation of sildenafil partially inhibits vasospasm in rabbits[4]; and 3) post-SAH initiation of sildenafil and other PDE5 inhibitors attenuates vasospasm in rat cisterna magna models of SAH.[568]

In the present study, we sought to not only confirm the anti-vasospasm effect of sildenafil in an endovascular perforation model of SAH, but also examine (for the first time) its mechanistic and functional consequences in the setting of SAH. First, we found that SAH increases PDE5 activity without altering PDE5 expression, and that sildenafil attenuates this SAH-induced augmentation of PDE5 activity. Two past studies have examined the effect of SAH on PDE5, with one showing increased PDE5 expression[7] and both showing increased PDE5 activity.[718] Our data corroborates that PDE5 activity is enhanced following SAH, but suggests post-translational modifications (rather than enhanced enzyme expression) is

primarily responsible. Next, we found that cGMP content in brain and cerebral vessels is reduced following SAH, and that sildenafil markedly but incompletely prevents this SAH-induced drop in cGMP (the incomplete restoration of cGMP might indicate that other cGMP-hydrolyzing PDE's are also contributing). Taken together, these data strongly suggest that enhanced PDE5 activity and subsequent augmentation of cGMP hydrolysis is responsible, at least in part, for SAH-induced downregulation of the NO-cGMP pathway. Moreover, we are the first to demonstrate that sildenafil attenuates SAH-induced downregulation of the NO-cGMP pathway by reducing PDE5 activity and restoring cGMP levels. A schematic illustration of these changes is shown in Figure 7.

Next, we examined several key questions regarding the therapeutic potential of PDE5 inhibition for SAH. First, we found that post-SAH initiation of sildenafil attenuates vasospasm in an endovascular perforation SAH model, which confirms previous cisterna magna injection SAH studies.[5–6, 8] Second, we document that sildenafil improves neurological outcome after SAH – a critical finding given that numerous studies have shown a dissociation between the vascular and functional benefits of certain SAH therapeutics.[21–22] Third, we found that sildenafil reduces SAH-induced neuronal cell death, which may reflect a reduction in vasospasm-induced ischemia or a direct neuroprotective effect (or both). Finally, we found that a therapeutically relevant dose of sildenafil does not cause systemic hypotension, does not affect cerebral vessel caliber in sham-operated mice, and does not exacerbate intracranial hypertension in SAH-operated mice.

PDE5 inhibitors have been approved by the Food and Drug Administration for the treatment of erectile dysfunction since 1998.[23] Over 100 manufacturer-sponsored trials of PDE5 inhibitors for the treatment of this condition have been completed, and an excellent safety profile has been consistently demonstrated.[24] Consistent with our expectations, sildenafil (2mg/kg) had no effect on BP or ICP in our experiments. In humans, PDE5 inhibitors have been associated with a slight reduction in BP[25]; however, this is rarely clinically relevant except in patients who are concurrently taking nitrates. Other reported side effects are either benign in nature or decidedly rare.[26]

Our study has several limitations. First, the endovascular perforation SAH model has been criticized for its high mortality and hemorrhage variability; however, it has been applauded for its acute pathophysiological changes that closely mimic aneurysmal rupture.[27–28] In our experience, mortality has been acceptably low (<10%). Also, we controlled for hemorrhage variability by distributing mice equally into different groups based on the severity of initial injury, before administration of vehicle or sildenafil. Second, rodent models of SAH do not completely recapitulate human SAH. Therefore, our findings regarding neuronal cell death, functional outcome, and side effects should be validated in other SAH models. Third, the improved neurological outcome noted in our study may reflect an activity of sildenafil other than its influence on vasospasm. For example, sildenafil inhibits platelet aggregation,[29–30] and could thus reduce formation of microthrombi, which have also been implicated in the pathophysiology of DCI after SAH.[31] Finally, the manner in which sildenafil reduces SAH-induced neuronal cell death was not addressed in our study.

Based on the findings that sildenafil prevents or reverses vasospasm following SAH in an endovascular perforation model in mice (this study) and a cisterna magna injection model in rats,[5] rabbits,[4] and dogs[7]; reduces neuronal cell death and improves neurological outcome after SAH in mice (present study); and causes no significant physiological side effects in mice subjected to SAH (present study); we conclude that sildenafil holds great promise as a strategy to prevent vasospasm and improve neurological outcome in patients with SAH. Though additional preclinical testing would be beneficial (e.g. to examine the effect of sildenafil on cerebral blood flow, neuronal cell death, and neurological outcome in larger animal models), we believe that the aforementioned excellent safety profile for sildenafil in humans indicates that a Phase I safety and dose-finding clinical trial examining sildenafil as a prophylactic strategy in patients with aneurysmal SAH is justified. Organization of such a trial is therefore currently underway.

Acknowledgements

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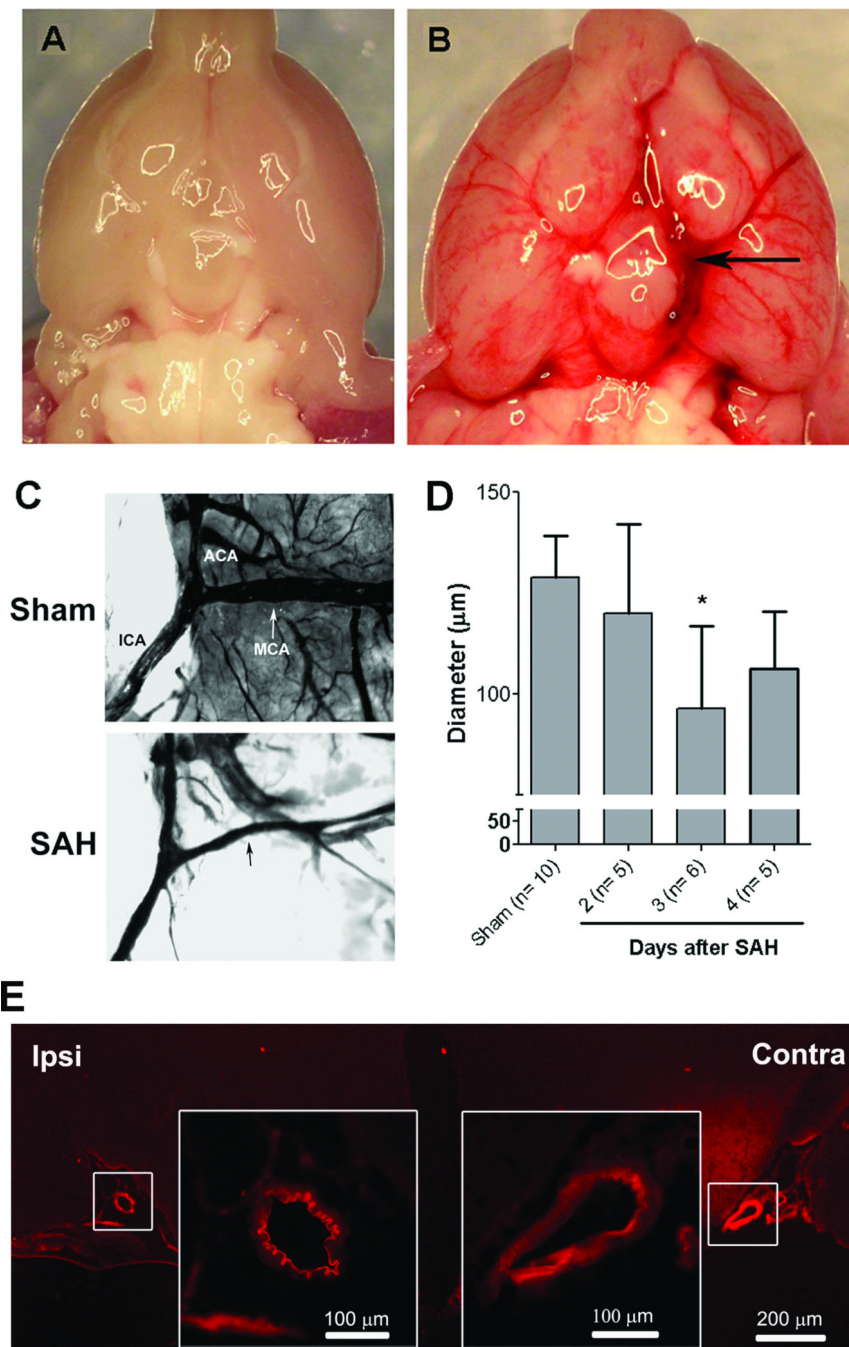
Figure 1. Cerebral vasospasm following experimental SAH in mice
Mice underwent sham operation or SAH by endovascular perforation of the left ACA. At 30 min post-surgery, ventral brain surfaces from mice subjected to sham (A) and SAH (B) were photographed. Extensive SAH centered at the site of arterial perforation was noted. On Days 2–4 post-surgery, brains from mice subjected to sham and SAH were cast with gelatin/india ink and imaged (C). Quantitation of MCA diameter demonstrated SAH-induced vasospasm peaked on Day 3 (D). Histological assessment on Day 3 also showed marked luminal

narrowing and intimal corrugation in cross-sections of cerebral arteries ipsi- but not contra-lateral to endovascular perforation (E).
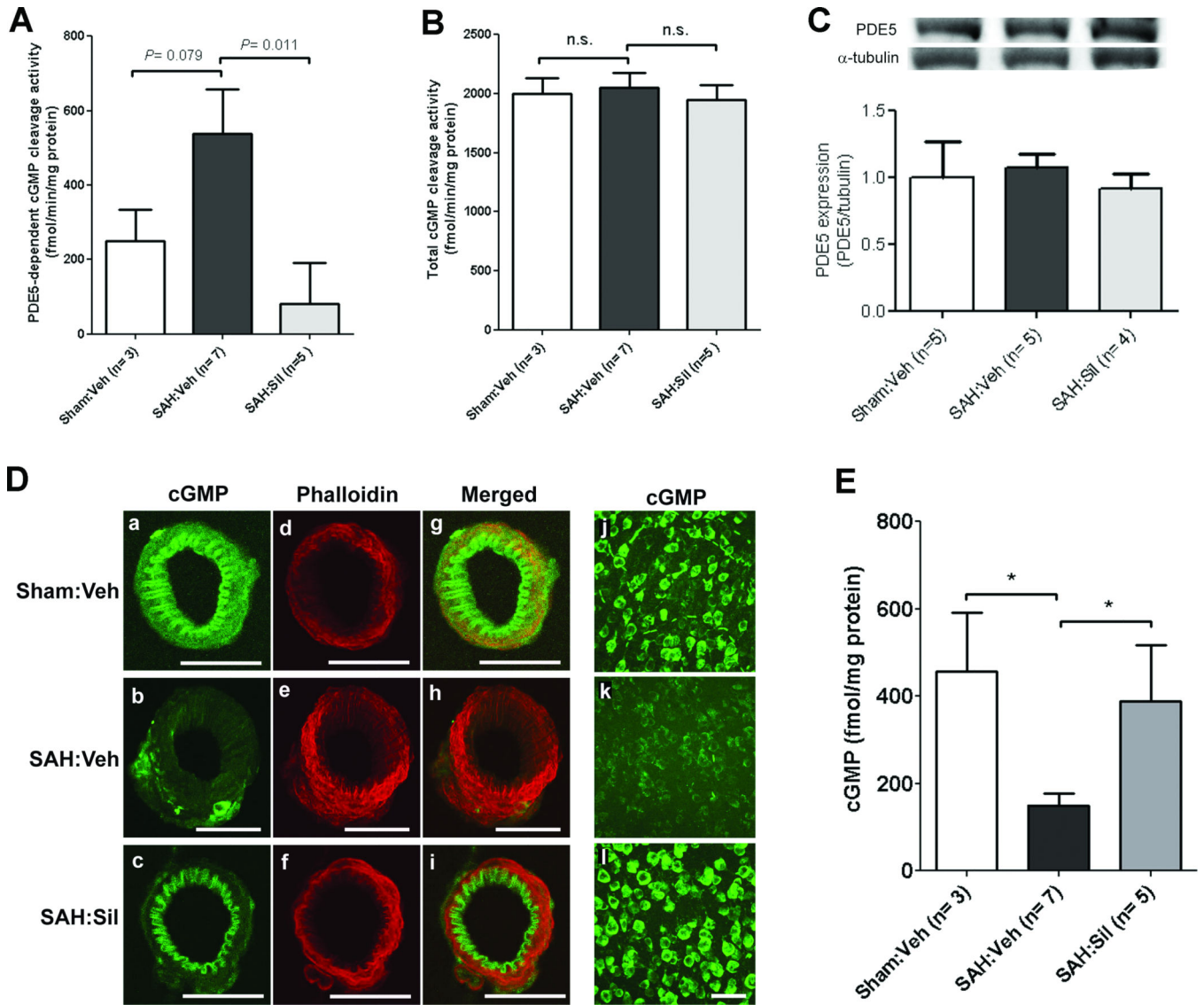
Figure 2. Sildenafil attenuates SAH-induced alterations in PDE5 activity and cGMP
Mice underwent SAH or sham operation. SAH mice were treated with vehicle or sildenafil (2 mg/kg P.O. BID) for 3d and brain tissues were prepared 3d post-SAH. Cyclic GMP-hydrolyzing activities were assayed with tissue lysates in the presence (A) or absence (B) of the PDE5 inhibitor zaprinast to determine the enzymatic activities of PDE5 (A) and total PDEs (B). Data indicate the mean±S.E.M. C. Representative western blot images and quantification data of PDE5 expression in the ipsilateral hemisphere. Expression levels of PDE5 were normalized to alpha-tubulin. Data indicate the mean±S.E.M. D. Representative images of MCA sections ipsilateral to SAH immunostained with cGMP (a–c) and co-labeled with the smooth muscle marker phalloidin conjugated with Alexa Fluor-568 (d–f) demonstrating that sildenafil attenuates SAH-induced drop in vascular cGMP. Superimposed images are also shown (g–i). Similar changes were seen in neuronal cGMP (j–l). Scale bar: 50 μm. E. Levels of cGMP in whole brains were determined by ELISA.

Data represent the mean±S.E.M. Statistics were analyzed by ANOVA followed by Newman-Keuls multiple comparison test.
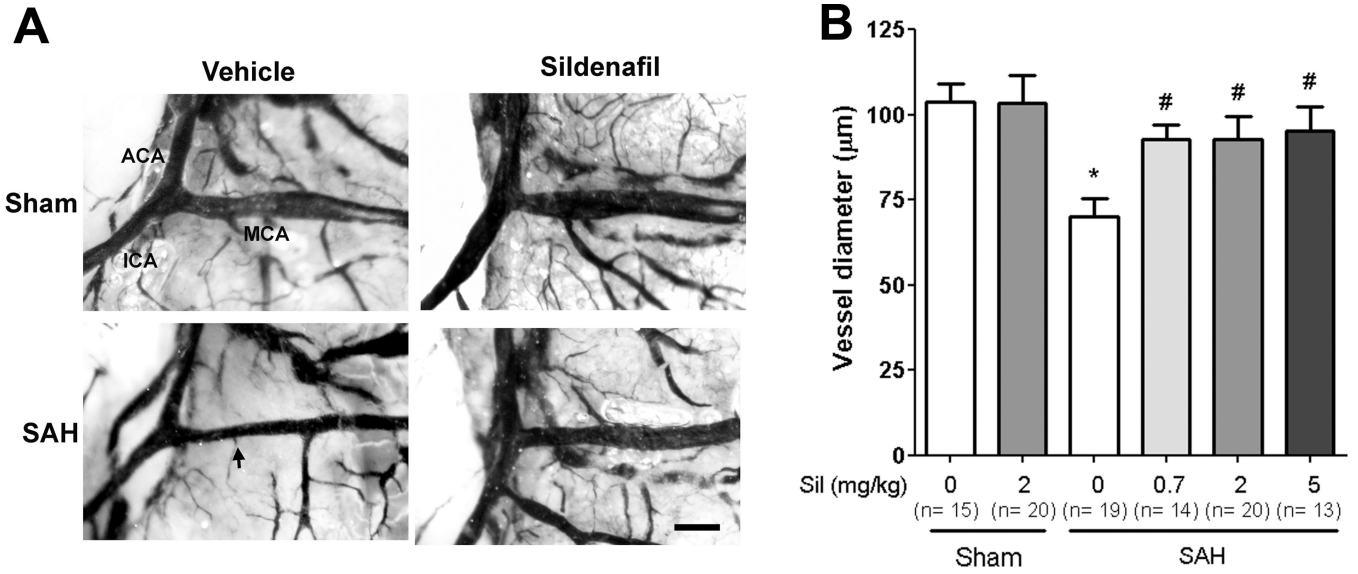
Figure 3. Sildenafil attenuates vasospasm following SAH

Mice underwent SAH or sham operation. Treatment with sildenafil (Sil) (0.7, 2 and 5 mg/kg P.O. BID) or vehicle (Veh) was initiated 2 hours later. On post-surgery day 3, mice were perfused with gelatin/india ink. A. Representative images of gelatin/India ink-casted cerebral vessels. Vasospasm of the MCA was evident in SAH mice compared with sham-operated mice; sildenafil attenuates this SAH-induced vasospasm. Scale bar: 200 µm. Abbreviations: ACA=anterior cerebral artery; ICA=internal carotid artery. B. Vessel caliber in the proximal MCA ipsilateral to suture perforation was determined. Data represent the mean±S.E.M. *P<0.05 vs. Sham:vehicle, #p<0.05 vs. SAH:vehicle. Data were analyzed by ANOVA followed by Newman-Keuls multiple comparison test.
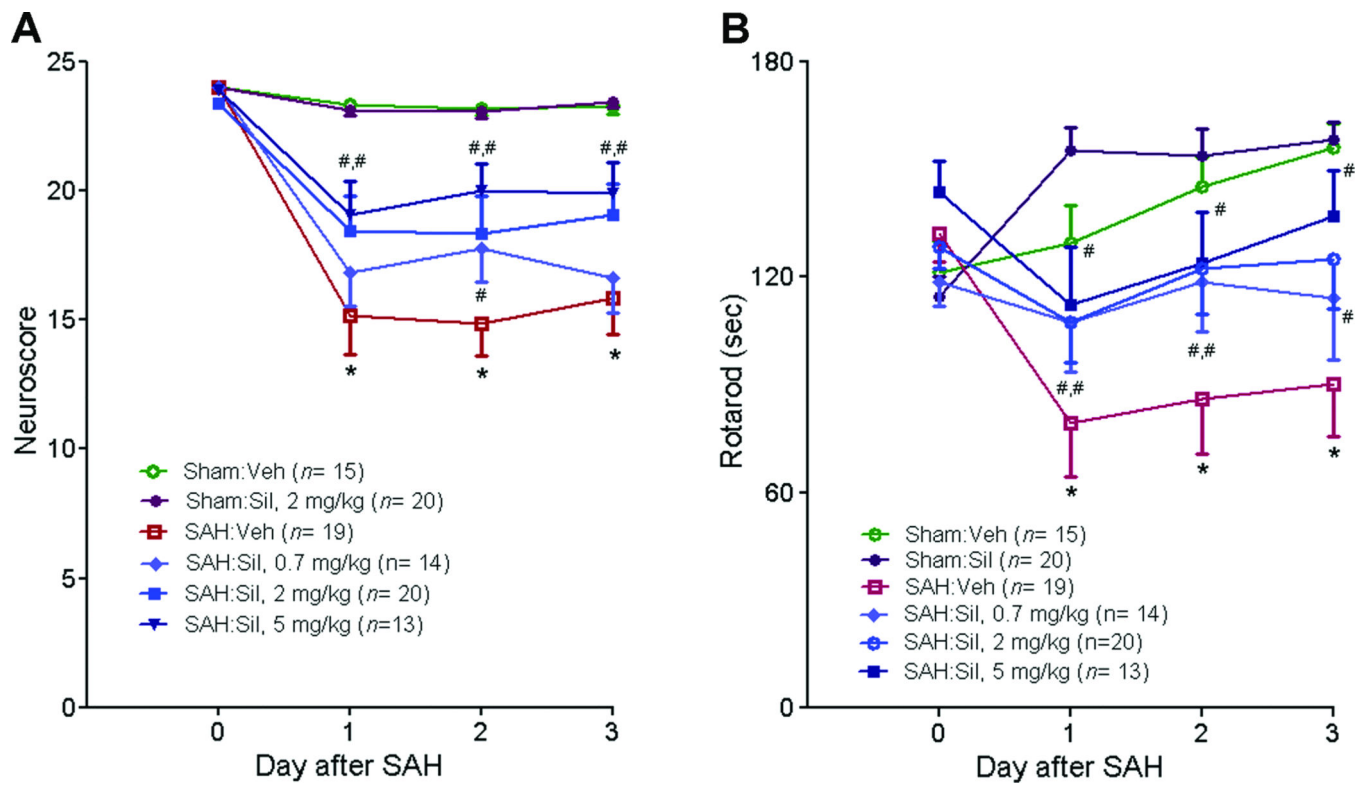
Figure 4. Sildenafil improves neurological outcome following SAH
Mice underwent SAH or sham operation. Treatment with sildenafil (Sil) (2 mg/kg P.O. BID)
or vehicle (Veh) was initiated 2 hours later. Behavioral performance was assessed daily via
sensorimotor neurological score (A) and rotarod latency test (B). Data represent the mean
±S.E.M. Statistical analysis was performed by Repeated Measures ANOVA followed by a
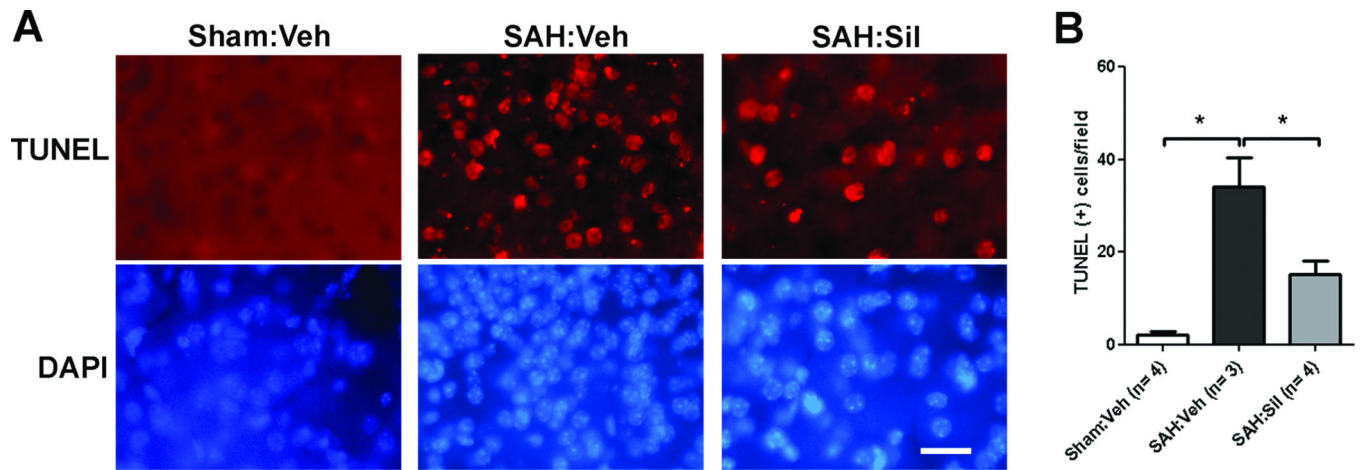*post-hoc* test.

Figure 5. Sildenafil reduces neuronal cell death after SAH
Mice underwent SAH or sham operation. SAH mice were treated with sildenafil (2mg/kg
P.O. BID) or vehicle (Veh); brain tissues were prepared 3d post-SAH. A. Brain sections
were labeled by TUNEL (upper panels) and stained with DAPI (lower panels). Scale bar:
25μm. B. Quantitative analysis of TUNEL positive cells in the ipsilateral parietal cortex per
field (83,083 μm^2). Data represent the mean ± S.E.M. *P<0.05 vs. Sham:vehicle, # p<0.05
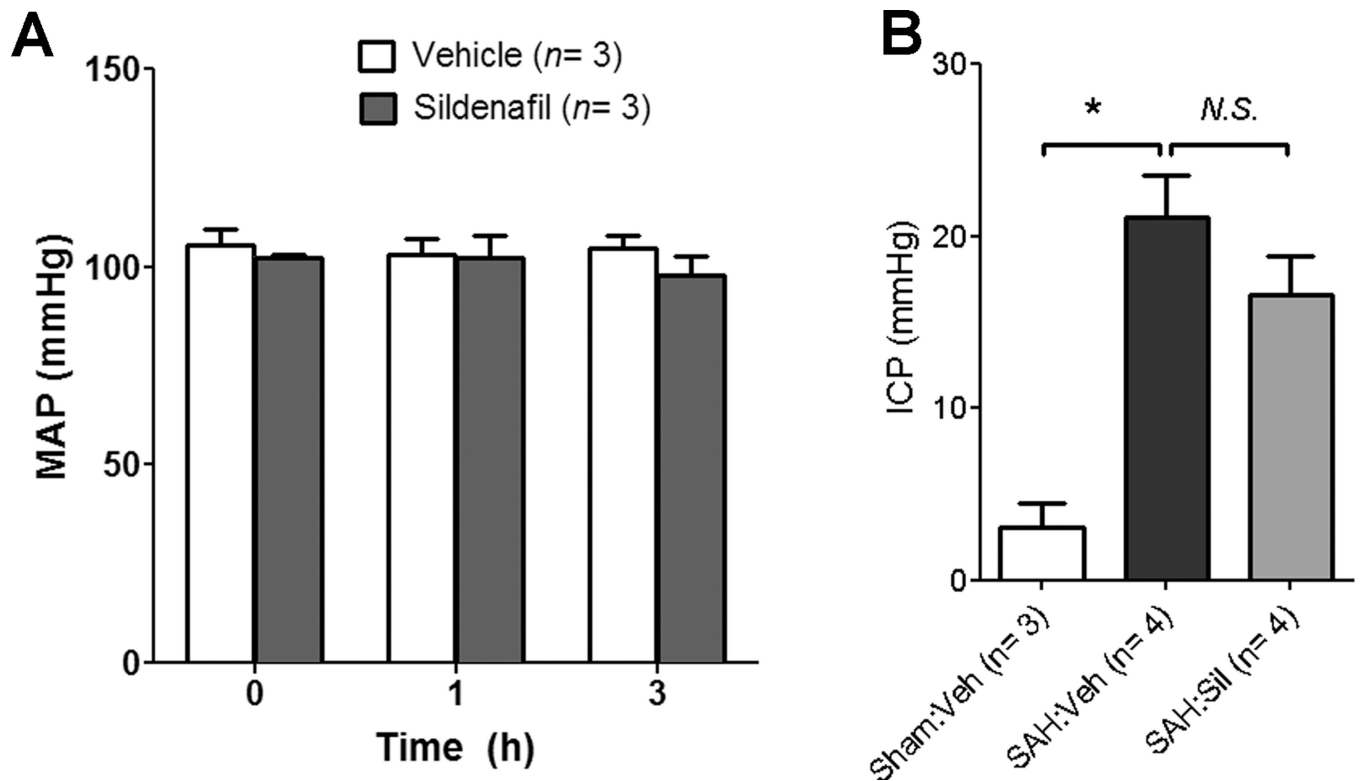vs. SAH:vehicle.

Figure 6. Sildenafil does not affect systemic blood pressure or intracranial pressure
A. Mean arterial blood pressure (MAP) was monitored via catheterization of the common carotid artery before and after treatment with sildenafil (Sil) (2 mg/kg P.O.) or vehicle (Veh) in normal mice. B. In a separate experiment, mice were treated with sildenafil (2 mg/kg P.O.) or vehicle 2 hours after SAH surgery. One hour later, ICP was monitored using a Mikro-tip pressure catheter. Data represent the mean±S.E.M and were analyzed by ANOVA followed by Newman-Keuls multiple comparison test. *P<0.05 vs. Sham:vehicle, #p<0.05 vs. SAH:vehicle.
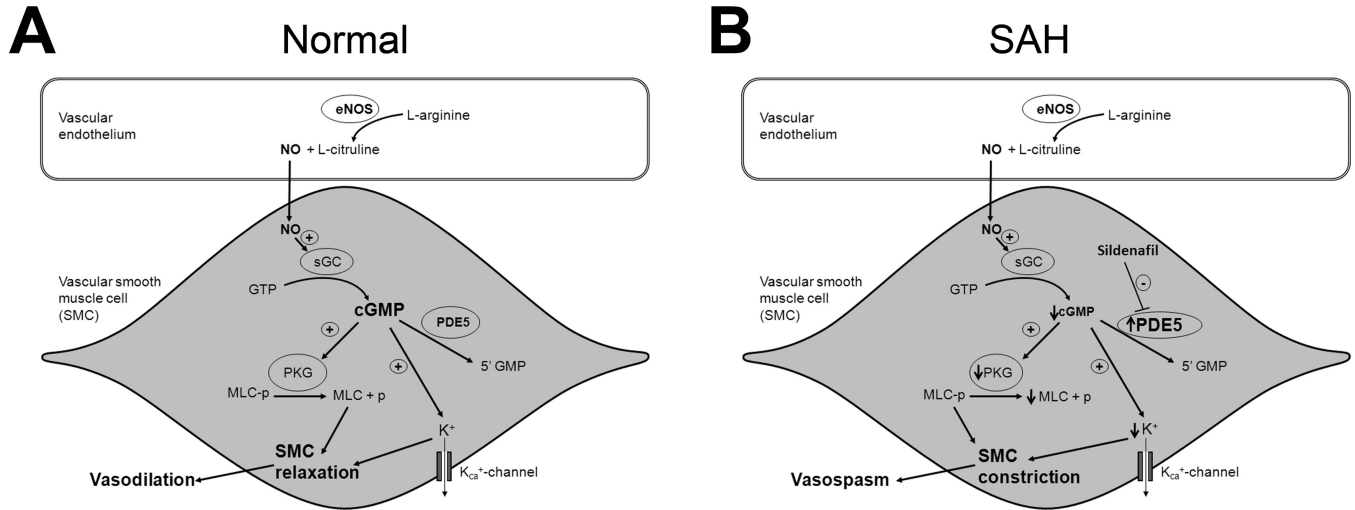
Figure 7. Schematic representation of NO-cGMP pathway
A. Under normal physiological conditions nitric oxide (NO) produced in the vascular endothelium diffuses into the vascular smooth muscle cells (SMC) and activates soluble guanylate cyclase (sGC). sGC enhances the conversion of guanosine-5'-triphosphate (GTP) to cyclic guanosine monophosphate (cGMP). cGMP activates a variety of downstream molecules including protein kinase G (PKG) and calcium dependent potassium channels (K_{Ca}^+). Activation of PKG leads to dephosphorylation of myosin light chain (MLC), and activation of K_{Ca}^+ leads to SMC hyperpolarization via potassium (K^+) efflux. Both these changes lead to SMC relaxation and subsequent vasodilation. B. Increase in phosphodiesterase-5 (PDE5) activity after SAH reduces cGMP levels. Decreased cGMP levels result in maintenance of phosphorylated MLC (via decreased PKG activity) and SMC depolarization (via decreased K^+ efflux) leading to SMC constriction and vasospasm. Inhibition of PDE5 with Sildenafil attenuates these pathophysiological changes and reduces vasospasm.