Effects of Topical Administration of Nimodipine on Cerebral Blood Flow following Subarachnoid Hemorrhage in Pigs

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Abstract

We sought to explore whether topical administration of nimodipine improves the abnormal cerebral perfusion following subarachnoid hemorrhage (SAH) in pigs. Fourteen pigs were randomly divided into three groups: sham ($n=4$), SAH ($n=5$), or SAH + nimodipine ($n=5$). The SAH model was established by injecting fresh autologous nonheparinized arterial blood into the suprasellar cistern. Nimodipine or saline placebo (0.04 g/mL) were administered to the operative area on the fourth day after the SAH model was established. The cerebral blood flow (CBF) was measured 60 min after topical administration of nimodipine by cranial SPECT/CT scans with 5 mCi ^{99m}Tc -ECD injected intravenously. The CCR (corticocebellar ratio) was calculated by dividing the counts/voxel of the whole cerebral hemisphere by the average count/voxel in the cerebellar region of reference and RD (relative dispersion). A predictor for impaired autoregulation of CBF was calculated by dividing standard deviation (SD) of regional perfusion by mean perfusion ($\text{RD} = \text{SD}/\text{Mean}$). CCR and RD were applied to describe hemisphere CBF and perfusion heterogeneity. Cerebral perfusion significantly decreased in the SAH group (CCR: 1.382 ± 0.192 , RD: 0.417 ± 0.015) compared to sham (CCR: 1.988 ± 0.346 , RD: 0.389 ± 0.015) ($p < 0.05$). Abnormal cerebral perfusion status, however, was not significantly improved in the nimodipine + SAH group (CCR: 1.503 ± 0.107 , RD: 0.425 ± 0.018) compared to the SAH group ($p > 0.05$). Topical administration of nimodipine did not significantly improve CBF following SAH. These findings were not consistent with our previous data demonstrating that the topical administration of nimodipine significantly alleviates cerebral vasospasm following SAH detected by TCD. Potential mechanisms governing these disparate outcomes require further investigation.

Key words: cerebral blood flow; nimodipine; pig; SPECT/CT; subarachnoid hemorrhage

Introduction

INCIDENCE OF SAH from rupturing intracranial aneurysms is about 6 to 8 per 100,000.¹ In the pathophysiologic evolution of intracranial aneurysms after rupture, brain hypoperfusion from delayed vasospasms, but not hydrocephalus or aggressive ICP increase, is the most dominant cause of morbidity and mortality.² Therefore, treatment against these vasospasms is crucial to ameliorate undesired prognoses.

Nimodipine is a dihydropyridine drug that blocks calcium influx through L-type calcium channels. It has been approved by the US Food and Drug Administration for use in vasospasm treatment and primarily administered orally and intravenously.² Even though this drug has produced promising results, drawbacks include hypotension, hypoxemia, and severe irritating discomfort. These side effects confine its usage.^{3–5} Illumination from intracranial cistern irrigation with papaverine after aneurysm clipping suggests that topical nimodipine administration following craniotomy may be more accessible to spasmodic pial arteries, and that a less systematic response is triggered.

This may be due to irrigation into the subarachnoid space or cistern directly, precluding any systematic circulation. Our previ-

ous data show that topical administration of nimodipine at 1:5 (0.04 mg/mL) and 1:10 (0.02 mg/mL) significantly alleviates cerebral vasospasm by TCD following SAH in rabbits.⁶ Vasospasm following SAH occurs in both the macrovasculature and microvasculature.⁷ The status of macrovasculature vasospasms diagnosed by TCD or DSA, however, does not correlate with cerebral blood flow (CBF). This is determined at the microvasculature level.⁸ Direct evidence regarding topical administration of nimodipine targeting microvasculature vasospasm to ameliorate CBF reduction following SAH still requires elucidation.

Methods

Animals and groups

Fourteen male mini-pigs weighing 10–15 kg each were randomly arranged into three groups: sham ($n=4$), SAH ($n=5$), and SAH + nimodipine ($n=5$). All procedures were approved by both the ethics committee of institutional animal care and the use committee of Shanghai Jiaotong University. Fourteen pigs received humane care in compliance with the “Principles of Laboratory

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Animal Care” formulated by the Shanghai Jiaotong University Committee of Medical Research, and “Guide for the Use of Laboratory Animals” issued by the Institute of Laboratory Animal Resources of Shanghai Jiaotong University.

SAH model

The SAH model in pigs was achieved as previously described.^{6,9} Each mini-pig was anesthetized with a bolus of SuMianXin, a commercial name for xylinidinothiazoline (JiLin University, Chang-Chun, China) at 1.2 mg/kg intramuscularly. The skin at the right side of the groin was incised to expose the femoral artery and vein for catheterization. Intravenous anesthesia in the form of 10 mg/mL propofol (AstraZeneca, Milano, Italy) was given at a 0.5 mL/kg/h rate. Arterial pressure and saturation of arterial blood oxygen was continuously monitored by MPA, Multiple Signal Analysis System (ALCBIO, Shanghai, China). Since propofol presented a low risk for apnea and adequate saturation of arterial blood oxygen during the operation was achieved, tracheal intubation remained unnecessary. Pigs were positioned at a lateral-prone posture, and a longitudinal cut was made between the right eye and ipsilateral ear. The scalps were retracted and the zygomatic arches resected. The incised temporal muscle vertical to the scalp was cut and retracted downwards to expose the anterior skull base. A bore with an approximate 2 cm diameter was drilled as low as possible to reach the anterior skull base. When the dura was opened and a 6F silicone rubber catheter was inserted along the anterior skull base, ipsilateral suprasellar cistern was confirmed by the outflow of transparent CSF from the catheter. The dura was tightly sutured. A 5 mL aliquot of autologous arterial blood (nonheparinized) from the arterial catheter was collected and immediately injected via injection pump from the extra-cranial tip into the suprasellar cistern within 1 minute. Sham group members were injected with 5 mL saline. The catheter was removed and the pore left of the dura mater was sutured. The wound was closed in layers and gelatin sponges replaced the removed skull bone.

Topical administration of nimodipine

Four days after the blood injection, pigs were anesthetized again by a procedure similar to the SAH model. Surgical sites were reopened before drug administration, and right frontal lobes were elevated by a flat and smooth metal board specialized for microsurgery to expose arteries at basal cisterns. A nimodipine dilution (0.04 mg/mL, 40 mL) from Nimotop (Nimotop® diluent, Bayer HealthCare Company, Germany) was carefully irrigated onto surfaces of both cerebral arteries and the brain within thirty minutes in the SAH + nimodipine group. Saline (40 mL) was applied to SAH or Sham group members.

SPECT/CT Scan

Scans were mainly completed by two neuroradiologists blind to the animal group arrangement. One hour after nimodipine or saline irrigation, each pig was scanned to acquire infused SPECT and CT images by a gamma camera (Philips Precedence SPECT/CT Camera System, ADAC Laboratories, USA). This camera acquires SPECT and CT images simultaneously from the same instrument, which is equipped with two 180° opposing rotating detectors with low energy and high-resolution collimators for full 360° rotation. Animals were placed in a dark, calm environment while 99mTc-ECD (5 mCi 185 MBq) was intravenously injected 30 min before the scan started. All pigs were placed at the same prone position with head-fixed in a device within the scanner. Before the SPECT scan, a CT scan was initially acquired (transaxial slices 2 mm), SPECT data was collected from 128 projections in a 128 matrix × 128 matrix, resulting in 6 to 8 million counts every 20 sec to acquire a projection. The SPECT images with a 7 to 8 mm resolution were saved on a hard disk for future analysis. After image acquisition, pigs were sacrificed by intravenously injecting 10 mL of 10% KCl to induce cardiac arrest.

Image analysis

Data collection was completed by a bioengineer guided by a neurosurgeon, both of whom were also blind to group arrangement.

Transaxial slices (thickness 2 mm) were reconstructed using an Astonish technique (Jetstream Workstation, Precedence Imaging System 2.0) for SPECT images. Chang's correction method of tissue attenuation was applied with a uniform attenuation coefficient of 0.12 cm^{-1} .¹⁰ Consecutive slices (thickness 2 mm) were obtained and prepared for ROI (regions of interest) analysis similar to McGoron's methods.¹¹ For the task of ROI research, a tri-planar volume Viewer and Analysis software MRicro 1.38 Build5 was employed. First, slices were rotated and realigned according to SPECT/CT images of a normal pig brain used as a template. Second, slices were spatially normalized to the template. Third, the ROIs were drawn manually on the registered transaxial slices of CT images for high spatial resolution and anatomy distinguishing ability. Registered CT ROI map slices correspond to SPECT slices because the acquisition was infused. All parameters were calculated by a program edited with commercial software, MATLAB 7.0 (The MathWorks, Natick, MA, USA).

For the right hemisphere total perfusion analysis, ROI presenting the right cerebral hemisphere were drawn on every slice. Another ROI was delineated at the ipsilateral cerebellar hemisphere as a reference region. Mean counts/voxel of right hemisphere were normalized to cerebellar tracer uptake with a CCR parameter (corticocerebellar ratio). CCR is a very applicable index to semi-quantify radioactivity uptake by the cerebrum and calculated by dividing the counts of the whole cerebral hemisphere by the average count per pixel in the cerebellar region of reference (right cerebral hemisphere counts/voxel vs. reference region of cerebellum counts/voxel).¹²

For the hemisphere perfusion heterogeneity calculation, which is a risk factor of impaired autoregulation of CBF and quantified by RD (relative dispersion) parameter, each slice (thickness 2 mm) was divided into 2 or 3 ROIs in right hemisphere.^{13,14} In the course of manual ROI drawing, the size of every ROI should be visually equal. Every right hemisphere was divided into more than 100 ROIs.

The RD was calculated from the mean counts/voxel in each ROI by dividing standard deviation (SD) of regional perfusion by mean perfusion ($\text{RD} = \text{SD}/\text{Mean}$).^{13,14}

Statistical analysis

Values were expressed as means ± SEM. Statistical significance between two means was determined by the Mann-Whitney test, and significance between multiple means was determined by the Kruskal-Wallis H test. $P < 0.05$ was considered statistically significant.

Results

Cerebral perfusion after SAH

The SAH model was successfully established and confirmed by CT scan (Fig. 1). CCR was 1.988 ± 0.346 and RD was 0.389 ± 0.015 in the sham group. Compared with sham, CCR significantly decreases to 1.382 ± 0.192 ($p < 0.05$) (Figs. 2 and 3), and RD increases to 0.417 ± 0.015 in the SAH group ($p < 0.05$) (Figs 2 and 4, Table 1).

Effects of topical nimodipine administration on cerebral perfusion

Comparing the two parameters (CCR and RD) of the SAH group with the SAH + nimodipine group, topical nimodipine administration does not statistically improve the decreased CCR

FIG. 1. CT scan on the fourth day after SAH shows that the blood collects within the suprasellar cistern, especially within right part of this cistern (arrow).

(1.503 ± 0.107) (Fig. 3) and increased RD (0.425 ± 0.018) ($p > 0.05$ for both). (Fig. 4, Table 1)

Discussion

In this study, we found that perfusion of the cerebral hemisphere significantly decreased and perfusion distribution heterogeneity increased on the fourth day after SAH. These data revealed the decline of CBF and impaired autoregulation of CBF in our model.

FIG. 3. The CCR values statistically decreased in SAH group compared with sham group. However, the CCT values was not significantly different between SAH and the SAH + nimodipine group (▲ $p < 0.05$, ● $p > 0.05$).

Furthermore, we have confirmed that the topical administration of nimodipine did not improve reduced perfusion and impaired CBF autoregulation following SAH.

The SAH model establishment and vasospasm confirmation

There are several SAH animal models: 1) intracranial artery is punctured allowing blood to outflow and congregate around the arteries; 2) intracranial arteries are exposed by craniotomy and autologous blood obtained from extracranial site is placed around the arteries within subarachnoid space; and 3) blood from another site is injected into the cisterna magna without craniotomy and collects around intracranial arteries. The optimal approach to

FIG. 2. SPECT images shows normal cerebral perfusion in sham group (A,B,C,D) and decreased cerebral perfusion on right hemisphere (E,F,G,H) in SAH group.

FIG. 4. The RD values statistically increased in SAH group compared with sham group. However, the RD values was not significantly different between SAH and the SAH + Nimodipine group ($\Delta p < 0.05$, $\bullet p > 0.05$).

induce vasospasm involve blood placement or injection, but blood injection via cisterna magna usually results in blood aggregation only around the brain stem or basilar artery. This deviates from a clinical situation.^{15–17} We chose the craniotomy approach and injected blood into the suprasellar cistern to establish an SAH model, similar to the one we constructed in rabbits.⁶

The definition for vasospasm directly implies a reduction in vessel caliber. When the spasm is induced by SAH, the underlying meaning becomes a limited definition as complexity is due to multiple factors: a delayed and reversible vasculopathy, impaired CBF autoregulation function, and hypovolemia bringing on a perfusion reduction in the supply territory of the affected vessel.² Vasospasm after SAH is mainly due to products from red blood cell degradation within the subarachnoid space and molecular induction from these products. This can relate to several pathophysiological mechanisms such as free radical or bilirubin oxidation product (BOX) formation, as well as protein kinase activation and receptor upregulation for vasoconstrictive substances.¹⁸ These potential mechanisms may share a common signal pathway, resulting in free Ca^{2+} overload in the cytoplasm of smooth muscle cells. Our previous data show that middle cerebral artery spasms were significantly relieved at 1 h after nimodipine irrigation, as detected by TCD. Moreover, 0.04 g/mL nimodipine solution had the most pronounced therapeutic efficacy in the rabbit SAH model. Unfortunately, we failed to find statistical significance of CBF between the SAH and SAH + nimodipine groups (0.04 g/mL nimodipine) by SPECT.

Given our results, it was difficult to explain the different results from data determined by TCD and SPECT. TCD is a specialized method used to test flow velocity of a major vessel and its branches, an example being the skull base artery. SPECT is optimized to determine CBF at the microcirculation level. When deliberately

probing the discrepancy between TCD and SPECT for vasospasm detection, disagreement has been reported in the literature.²⁰ Traditionally, scientists have focused on macrovasculature spasms owing to SAH. In recent years, microcirculation studies have been in the forefront of vasospasm pathophysiology research. Microcirculation dysfunction in SAH has been explored for decades. Its characterization includes decreased CBF, aggregation of red blood cells, and reduction of arteriole and venule diameter.^{21,22} Approximately 40% of SAH patients with angiographic vasospasms are spared from neurological defects, while some SAH patients with neurological symptoms do not exhibit angiographic vasospasm.²³ Thus, microcirculation dysfunction is beyond the scope of angiography, and TCD should explain this discrepancy. Moreover, other studies have revealed no correlation that exists between regional CBF decrease and lumen reduction of large arteries supplying this region.²⁴ All of these references have addressed dissociation between macrovasculature spasms and microvasculature dysfunction. In our present model, we report evidence for vasospasms at the microvasculature level by SPECT, taken as ‘snapshots’ to detect cerebral perfusion of microcirculation.⁷ CCR (corticocerebellar ratio) calculated from our experimental data, the semi-quantity parameter for hemisphere radioactivity uptake proportional to CBF, can allege microvasculature spasms.⁷ Additionally, heterogenous perfusion distribution manifested by enlarged RD is a predictor for impaired autoregulation of CBF, and emphasized vasospasm occurrence in our model.^{13,14}

Nimodipine effects on vasospasm and CBF after SAH

Nimodipine usage for vasospasm treatment has been supported by other studies. Nimodipine is administered either orally or intravenously. In a prospective randomized aneurysm nimodipine trial, an oral bolus of 60 mg nimodipine was given every 4 h for 3 weeks, beginning no more than 4 days from SAH ictus. This regimen was proved to reduce infraction by 34% compared to the placebo group.¹⁷ Intravenous infusion was also reported to reduce ischemia secondary to SAH.²⁶ Another prospective randomized clinical trial reported no difference in ischemia prevention and prognosis improvement between intravenous and oral nimodipine administration.²⁷ Recently, reports involving intra-arterial nimodipine infusion, mostly optimal for endovascular treated patients with SAH, highlighted promising advantages regarding this approach.²⁸

Compared to intravenous or intra-arterial approaches, topical administration into the cistern or intrathecal instillation is relatively rare. In Auer’s study, nimodipine solution (2.4×10^{-5} M) was applied to exposed intracranial arteries of 17 patients intraoperatively within 72 hours after aneurysm rupture. Postoperatively, 15 patients (88%) were spared from symptomatic vasospasm, and the DSA did not show any severe spasms after 1 week after application, as had been previously highlighted for this measure.²⁹ Studies were conducted to validate that nimodipine irrigation can improve neurological outcomes.³⁰ Although most studies reached an agreement that nimodipine can alleviate vasospasm, they were confined to basilar artery diameter determination. We are the first group to determine the effects of topical irrigation of nimodipine on CBF by SPECT and show that it cannot increase cerebral hemisphere perfusion at 1 h after drug administration. Our findings are consistent with a report showing that nimodipine infused intrathecally by an ommaya pump (1 mL/0.2 mg tid) for 6 days had no apparent effect on spasmodic basilar artery caliber dilation.¹⁵

TABLE 1. THE COMPARISON OF CCR AND RD FOR THREE GROUPS

Group	Corticocerebellar ratio CCR	Relative Dispersion RD
Sham	1.988 ± 0.346	0.389 ± 0.015
aSAH	1.382 ± 0.192^a	0.417 ± 0.015^a
aSAH + nimodipine	$1.503 \pm 0.107^{a,b}$	$0.425 \pm 0.018^{a,b}$

^a $p < 0.05$ versus Sham group; ^b $p > 0.05$ versus aSAH group.

Nimodipine has been shown beneficial to SAH, and can also ameliorate angiographic vasospasms.³¹ However, Pickard pointed out that the beneficial effects of nimodipine on neurologic outcomes in SAH patients did not correlate with angiographic presence or absence of cerebral vasospasms prior to therapy.³² Definite therapeutic effects on microvasculature following SAH for CBF elevation remain elusive.^{31,33} Our negative results and conclusion regarding the effects of nimodipine on CBF is consistent with other reports. Nimodipine did not prevent the reduction of regional CBF after SAH by a microspheres method in an experimental study. It was presumed that nimodipine infusion only increases CBF in normal subjects but not in SAH ones.³⁴ A clinical study performed to investigate the effects of nimodipine on autoregulation of CBF using the 133-Xenon method also confirmed nimodipine does not significantly improve CBF in severe SAH patients.³³ Other approaches of nimodipine administration have also been evaluated. Hänggi showed that intra-arterial administration of nimodipine (IAN) only has temporary efficacy in increasing CBF. He highlighted that even though IAN can increase cerebral perfusion as detected by CT perfusion, this potential lasted no more than 24 hours after intervention and could not persist during the following days.³⁵ An encouraging opportunity to improve CBF was the intrathecal nimodipine infusion trial. This trial, however, lacked a control group and includes many other antispasm interventions, including intravenous or intra-arterial infusion of Nimodipine, haemodynamic treatment or balloon angioplasty, were applied simultaneously. This impaired the effects of evaluating intrathecal infusion exclusively.³⁶ Nevertheless, another medicament magnesium sulfate, replacing calcium ions, has been used to improve CBF of SAH rats by intracisternal injection (1.5 mL/min for 30 min).³⁷

Explanation for our results

Even though no definite explanation exists to explain why nimodipine failed to ameliorate microvasculature dysfunction due to SAH, we explored plausible reasons that may explicate this phenomenon. Properties of contractile activity may differ between large cerebral arteries and small vessels. These may be attributed to calcium channel specificity, and innervations or organization of smooth muscle are taken into consideration.³⁸ For instance, Ishiguro et al. observed constrictions are greatly enhanced in small cerebral arteries obtained from a rabbit model of SAH due to the emergence of R-type voltage-dependent calcium channels in arterial myocytes. Interestingly, nimodipine can antagonize L-type voltage-dependent calcium channels.³⁹ Small artery or capillary vessel spasms exhibit more dependence on non-L-type voltage-dependent calcium channels, and the effects of nimodipine on these targets may be more obscure. Certain noncalcium channel-dependent pathways contribute to vasospasm due to SAH.⁴⁰ This may explain why vessel constriction might be spared from drug effects. Scientists found, as early as 3 days after SAH ictus, pathological hyperplasia of intraparenchymal vessels, while extraparenchymal vessels upheld normal morphology to form the structural factor against nimodipine at the microvasculature level. This may have prevented functional caliber reduction. From this histological observation, the discrepancy of nimodipine's effects on the microvasculature and macrovasculature may be partially explained.⁴¹ Additionally, Zumkeller noted that nimodipine may damage the blood-brain barrier by disturbing CBF autoregulation, but the mechanism remains unknown.⁴² Our observation in this study coincides with other sources, and further study is certainly necessary to reveal underlying mechanisms of these results.

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Author Disclosure Statement

No conflicting financial interests exist.

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