Magnetic Facial Nerve Stimulation in Animal Models of an Acutely-Ruptured Cerebral Artery

Authors: Emilio Sacristan, Ph.D. 1,2,4
Cesar Garcia-Benitez 1
Andrea Garcia, M.S. 1
Dawn M. Bielawski, Ph.D. 3
Chisa Yamada, M.D. 3
Joaquin Azpiroz, Ph.D. 1
Mark K. Borsody, M.D., Ph.D. 2,3

Affiliations: 1 National Center for the Investigation of Imaging and Medical Instrumentation
Universidad Autónoma Metropolitana, Mexico City, Mexico
2 Nervive, Inc. 526 S. Main St., suite 801-A, Akron OH 44311
3 NeuroSpring 35756 Foothills, Sterling Heights, MI 48312
4 author for correspondence: esacristan@ci3m.mx, TEL: + 52 55 6729 2774

Running Title: Facial Nerve Stimulation After Cerebral Artery Rupture
Number of Words, Total: 3366

Number of Words, Abstract: 248

Number of Figures: 4

Number of Tables: 1

Key Words: facial nerve, magnetic stimulation, subarachnoid hemorrhage

Subject Codes: intracranial hemorrhage, neurostimulation, autonomic nervous system, basic science research
ABSTRACT

Background: We are developing an emergency treatment for ischemic stroke based on pulsed magnetic stimulation of the facial nerve, which is known to rapidly dilate cerebral arteries and increase cerebral blood flow. Because our device is non-invasive and easy to use, it might be useful in hyperacute or resource-limited settings. However, ischemic and hemorrhagic subtypes of stroke cannot be readily distinguished in such settings, and so our device might be unintentionally administered to hemorrhagic stroke patients.

Methods: In order to assess the safety of magnetic facial nerve stimulation delivered in the presence of brain hemorrhage, we developed a model in which a basal cerebral artery is punctured via craniotomy, causing subarachnoid hemorrhage. Three dogs were used in pilot experiments and 10 pigs in confirmatory experiments. The model was assessed according to the following physiologic measures: hematoma volume, intracranial pressure, and brain perfusion index. Facial nerve stimulation was delivered after hemorrhage at parameters that induce cerebral artery dilation and increase cerebral blood flow in normal animals and animals with ischemic stroke.

Results: Our model created subarachnoid hemorrhage with intraparenchymal extension of the hematoma. There was short-term enlargement of the hematoma and an immediate increase in intracranial pressure. Facial nerve stimulation did not further increase hematoma volume nor increase intracranial pressure outside of the stimulation period, but it did reverse a transient reduction of cerebral blood flow.

Conclusions: These results validate our model and demonstrate the general safety of facial nerve stimulation in the presence of an acutely-ruptured cerebral artery.
INTRODUCTION

Hemorrhagic stroke accounts for 15% of all stroke in the U.S. but perhaps as much as 50% of all stroke in other parts of the world [1]. At the onset of hemorrhagic stroke, intracranial pressure rises dramatically as the hematoma grows. Patients with hemorrhagic stroke are also more likely to have neck stiffness, seizures, and headache at the onset [2], yet assessment tools based on symptoms and clinical signs are not as reliable as neuroimaging at detecting hemorrhagic stroke [3]. However, in many parts of the world, neuroimaging is simply not available, and decision analysis models estimate a net clinical benefit of early administration of treatments for ischemic stroke to ‘undifferentiated’ stroke patients in resource-limited settings despite the higher rates of hemorrhagic stroke [4]. A similar analysis might suggest the benefit of earlier intervention in the undifferentiated stroke patient population with a new treatment for ischemic stroke that is safer in hemorrhagic stroke patients than the currently available standard-of-care.

We aim to develop an emergency treatment for ischemic stroke that can be used in resource-limited or hyperacute settings. Our device is based on a well-known phenomenon that stimulation of the autonomic components of the facial nerve dilates cerebral arteries and increases cerebral blood flow (CBF). This effect of invasive electrical stimulation of the facial nerve has been repeatedly demonstrated in normal animals [5-18]. Additionally, facial nerve activity maintains resting CBF levels [19] and is critically involved in CBF changes induced by other neural systems [20]. Furthermore, electrical stimulation of the facial nerve improves brain perfusion, reduces brain injury, and improves neurological function in animals with brain ischemia [21-24]. Indeed, one company (BrainsGate) is midway into pivotal trial testing of an invasive facial nerve stimulator device as a treatment for ischemic stroke. Developing upon this body of knowledge, we have shown that pulsed magnetic energy generated by a proprietary device [25] can similarly activate the autonomic components of the facial nerve in normal animals with results comparable to invasive electrical stimulation [26]. Subsequently, we demonstrated the effectiveness of this technology in an autologous blood clot embolism ischemic stroke model in dog, published previously in Stroke [27,28]. Because we intend our non-invasive device to be simple to apply in a manner based only on cranial anatomy, it may be readily administered in resource-limited and hyperacute settings.

Based on pilot experiments in dog, we developed a pig model of an acutely-ruptured cerebral artery so as to assess the safety of magnetic facial nerve stimulation should our device be unintentionally applied in that condition. Both animals have relatively large head sizes that are favorable for experiments with magnetic stimulation devices, but we focused our efforts on pig because periods of only 2 minutes of magnetic facial nerve stimulation have been shown to increase CBF for up to four hours in pig (manuscript submitted for publication). Any safety concerns of the stimulation should thus be emphasized in the pig because it appears to be highly responsive to facial nerve stimulation.
MATERIALS AND METHODS

A. Experimental Animals

The experimental protocol was performed with the approval of the ethics committee of the National Center for Medical Imaging and Instrumentation Research (C3M) and the Universidad Autónoma Metropolitana of Mexico City. The experimental protocol is essentially identical to that used in pilot dog experiments previously reported in Stroke [27]. Results from both types of animals are reported herein.

Three adult male mongrel dogs, specially-bred for research purposes, weighing 15-37 kg were kept with ad libitum access to food and water until 8 hours before the experimental procedure, at which time they were food restricted. Anesthesia was induced with intramuscular injection of Zoletil (tiletamine / zolazepam 1:1, 7 mg / kg), propofol (2.5 mg / kg), and fentanyl (2 μg / kg), and anesthesia was maintained with propofol (10 mg / kg / hr). Dogs were intubated after induction and mechanically ventilated without using neuromuscular paralysis. The femoral vein was catheterized for the delivery of MRI contrast agent.

Ten adult male Yorkshire breed pigs between three to six months of age and 10-20 kilograms bodyweight were reared under controlled feeding. Pigs were allowed ad libitum access to food and water until 8 hours before the experimental procedure, at which time they were food restricted. Pigs were induced using intramuscular dose of azaperone (2mg / kg) and ketamine (15mg / kg), and then were intubated and mechanically ventilated without paralytic agents. Isoflurane (1-2%) with 100% oxygen (3.2 L / min) was used for the maintenance of anesthesia. The lateral auricular vein was catheterized for delivery of MRI contrast agent.

In both dogs and pigs a catheter was placed in the femoral artery to record blood pressure and heart rate, and to obtain arterial blood gas samples. Ventilation was titrated according to oxygen saturation, end-tidal CO₂ levels, and arterial blood gas measures obtained hourly.

B. Arterial Rupture

Arterial rupture was caused by inserting a Tuohy needle under neuronavigation guidance (Brain Science Tools, Netherlands) through a 10 mm burr hole in the skull. In dogs, the target was the ipsilateral internal carotid artery, whereas in pigs the target was the left M1 segment of the
middle cerebral artery. Puncture of the target artery was immediately confirmed by pulsatile backflow of bright red blood through the Tuohy needle.

After induction of hemorrhage, the Tuohy needle was removed and the burr hole was sealed. In dogs, the burr hole was sealed with a patch of temporals muscle and the incision was sutured closed. In pigs, the burr hole was sealed with a custom-built plastic intracranial pressure monitoring screw that allowed for continuous monitoring of intracranial pressure.

All animals were sacrificed under anesthesia by potassium chloride injection and the brain was then removed for gross confirmation of middle cerebral artery rupture and subarachnoid hemorrhage.

C. Imaging and Facial Nerve Stimulation

MRI scans were obtained using a Philips Achieva 3T MRI scanner and an 8-channel SENSE coil. T1W and T2W scans of the whole head were obtained using a 3D Fast Gradient Echo sequence. The same geometry was used for the perfusion imaging and the T2 reference scans, with 25 coronal slices spanning the entire brain with a 230 mm FOV. T2 images used a multi-slice Fast Spin Echo sequence. Perfusion imaging used the Philips PRESTOs sequence consisting of 40 dynamic phase contrast images per slice repeated every 1.6 sec, obtained after a 3 mL bolus contrast agent (gadopentate dimeglumine 0.5 mmol / mL) followed by a 20 mL saline flush [29,30].

All animals were restrained on a specially-designed board so that they could be transferred in and out of the MRI scanner and always placed in the same position. In the pig experiments, MRI studies at five consecutive time points were acquired:

1) pre-hemorrhage baseline
2) immediate post-hemorrhage (PH0)
3) 60 minutes post-hemorrhage (PH60)
4) immediate post-stimulation (PS0)
5) 60 minutes post-stimulation (PS60).

Facial nerve stimulation was delivered with a fluid-cooled 6.5 cm figure-8 coil (MagVenture R30) ipsilateral to the ruptured artery and at the following parameters: biphasic 280 μsec pulses delivered at 10 Hz continuously for 5 minutes. In dogs, pulse power was set at 1.8 Tesla at coil surface, whereas in pig the pulse power was set at 1.6 Tesla at coil surface; the latter reflects
effective stimulation parameters defined for that species in currently unpublished data. The coil was fluid cooled so as to maintain surface contact temperatures < 40°C. The coil was placed against the head using neuronavigation guidance with the target being the geniculate ganglion region of the facial nerve, as previously described [27,28]. Sham stimulated animals were subject to the same coil positioning procedures but no active stimulation was delivered. Stimulation or sham stimulation was delivered immediately after completion of the MRI study performed 60 minutes after hemorrhage (i.e., after the PH60 time point). Stimulation or sham stimulation was administered according to coin toss allowing for forced allocation to balance the groups.

D. Digital Image Processing and Data Analysis

Perfusion index maps were calculated using the PRESTO® Philips software. Twenty-five maps were calculated for each slice of the perfusion scan. However, for quantitation of the perfusion index, only five central hematoma slices were used. The selected maps were exported to OsiriX® and the brain was delimited and segmented by using ImageJ® and Matlab®. The T2W reference scans were used to create an image mask and the brain was then segmented with SNAKE (Active Contours) [31-33]. The SNAKE was manually initialized by ROI in each HUANG filter threshold T2W image. The five slices were used to calculate the mean perfusion index at each time point that was normalized against the perfusion index measure of the pre-stimulation baseline for each animal.

Hematoma volume was determined on T2W imaging. We took the center slice, with the maximum hematoma area, and delimited and segmented the hematoma in a coronal section. Statistical comparisons were made on the pig data comparing stimulation and sham stimulation groups using repeated-measures ANOVA. Significance was set as P < 0.05.

RESULTS

No animal died during the course of these experiments either as a result of the hemorrhage or as a result of the stimulation. Hemorrhage was confirmed in all animals in the operating room prior to neuroimaging and then again by baseline neuroimaging that uniformly showed subarachnoid hemorrhage with intraparenchymal extension into the track left by the Tuohy needle (FIGURE 1).
The pilot experiments in dogs demonstrated that the hematoma volume generally stabilized within 30 minutes of hemorrhage onset. Thereafter, the hematoma slowly disperses. Similarly, in the pigs, the hematoma volume stabilized within 60 minutes of hemorrhage onset and slowly began to disperse thereafter (FIGURE 2). Also in the pigs, intracranial pressure was found to be increased above 50 mmHg immediately upon placement of the intracranial pressure monitoring screw; intracranial pressure subsequent stabilized between 20-30 mmHg without pharmacological intervention or cerebrospinal fluid drainage within 30 minutes from hemorrhage onset (FIGURE 3). Blood pressure, respiratory rate, and arterial blood gas measures were stable throughout the experiment (TABLE 1). Furthermore, a transient decrease in the cerebral perfusion index was observed (FIGURE 4).

In the pilot dog experiments, facial nerve stimulation did not appear to increase the hematoma size as assessed by qualitative comparison of the MRI images. Brain perfusion index also did not appear to increase as a result of stimulation in the dog experiments when the entire head of the animal was qualitatively examined in cross section (as reported in [27]). However, when the brain perfusion index was specifically evaluated and the range of the perfusion index restricted to that relevant to the brain (i.e., the extracranial tissues were masked), an increase in perfusion index became evident in the dogs (FIGURE 1).

In 5 pigs, stimulation of the facial nerve immediately following the MRI study performed 60 minutes after hemorrhage onset (PH60 time point) did not cause enlargement of the hematoma volume (FIGURE 2) nor did it increase intracranial pressure outside of the period of active stimulation (FIGURE 3). However, in the pig experiments, a transient increase in the perfusion index was observed immediately after stimulation in comparison to a control group (FIGURE 4). Cardiovascular, respiratory, and arterial blood gas measures were not affected by facial nerve stimulation in either dog or pig experiments (TABLE 1).

DISCUSSION

We demonstrate the technical feasibility of inducing intracranial hemorrhage by means of a neuronavigation-guided puncture of the internal carotid artery or proximal middle cerebral artery. The model was technically feasible in both dog and pig, and it exhibited features consistent with the human condition of subarachnoid hemorrhage, namely a sudden increase in intracranial pressure related to hematoma growth followed by accommodation to the intracranial mass and a slow dispersion of the hematoma. Furthermore, when quantification of CBF is employed as it was in the pig experiments, a small decrease in CBF caused by the hemorrhage became evident. Indeed, this may reflect the acute reduction of CBF that has been reported in
animals and man after subarachnoid hemorrhage [34], which may reflect a diffuse and transient vasospasm reaction [35,36].

The facial nerve has been shown to dilate the arteries of the brain and increase CBF in normal animals [6-20], and electrical stimulation of the autonomic components of the facial nerve reverses several measures of brain ischemia in animal models of ischemic stroke [21,22] and delayed cerebral artery vasospasm following subarachnoid hemorrhage [22,23]. Our own experiments with non-invasive facial nerve stimulation using pulsed magnetic energy confirm these observations [26-28]. Intuitively, one might think that dilation of a ruptured cerebral artery in this manner would lead to rehemorrhage. However, this is not a well-substantiated assumption. Dilation of the cerebral arteries after facial nerve stimulation occurs by relaxation of the arterial smooth muscle that would reduce – not increase – the tension on the arterial wall. Reduced arterial wall tension would logically be reflected across a blood clot that seals the puncture site, which otherwise would not be involved in the change in arterial caliber. Furthermore, since facial nerve stimulation does not affect cardiovascular performance, dilation of the cerebral arteries by facial nerve stimulation should reduce vascular resistance, thereby reducing local blood pressure and sheer stress at the puncture site.

Herein we confirm our previous finding in dogs with an acutely-ruptured middle cerebral artery that were published in Stroke [27], namely that magnetic facial nerve stimulation does not increase hematoma volume. We further report that intracranial pressure was only transiently increased to a small degree during stimulation, an effect that is likely due to vibration of the head induced by facial muscle contractions [37]. Studies of invasive electrical stimulation of the sphenopalatine ganglion have also shown that intracranial pressure is not increased in a monkey model of delayed cerebral artery vasospasm caused by surgical implantation of blood clots in the subarachnoid space [24]. While such an animal model would not allow for stimulation-induced hemorrhage, it does demonstrate dilation of the cerebral vasculature without an increase in intracranial pressure.

We next must confirm the safety of magnetic facial nerve stimulation in a larger group of animals with this model of cerebral artery puncture. Additionally, a companion study is planned for intracerebral hemorrhage using the established intraparenchymal collagenase pig model [38]. Safe use of a non-invasive, easy-to-use facial nerve stimulation device would potentially allow the device to be emergently applied to undifferentiated stroke patients prior to, or in lieu of, diagnostic evaluation to rule-out hemorrhagic stroke, as would be needed for hyperacute stroke treatment and/or treatment in resource-limited settings. The improved time-to-treatment and clinical outcomes for ischemic stroke patients that could be achieved by treatment of the undifferentiated stroke population in this manner could then be considerable.
SUMMARY / CONCLUSIONS

Our animal model of an acutely-ruptured middle cerebral artery exhibited key features of clinical subarachnoid hemorrhage in man. Stimulation of the facial nerve non-invasively with pulsed magnetic energy did not cause enlargement of the hematoma or a prolonged increase in intracranial pressure, but nevertheless it reversed a transient reduction in CBF.
ACKNOWLEDGMENTS

This research was supported by the National Center for Medical Imaging and Instrumentation Research of the Metropolitan Autonomous University of Mexico City and by NeuroSpring, a not-for-profit organization that supports neuroscience research (www.neurospring.org).

SOURCES OF FUNDING

This research was supported by a generous grant from the Bugher Foundation.

CONFLICTS OF INTEREST

Drs. Borsody and Sacristan are shareholders in, and serve in senior leadership roles for, Nervive, Inc., which is developing the facial nerve stimulator technology. The other authors have no conflict of interest to disclose.
REFERENCES


2. Ruchey S, McGee S. Does this patient have a hemorrhagic stroke? *JAMA* 2010; 303: 2280-6.


Figure 1: An example of internal carotid artery puncture in dog demonstrating the effect of facial nerve stimulation on the hematoma (white arrow) and perfusion index. Top panel, gradient T2 images; bottom panel, perfusion index. Immediate post-hemorrhage (PH), immediate post-stimulation (PS).
Figure 2. Hematoma volume, demonstrating a lack of effect of facial nerve stimulation compared with no stimulation. Pig experiments, n=5 per group. Hematoma volume normalized to that measured 60 minutes post hemorrhage (PH60).
Figure 3. Intracranial pressure measured by subdural catheter in facial nerve stimulated group and sham stimulated group every 5 minutes. Pig experiments, n=5 per group. P<0.05 by repeated-measures ANOVA.
Figure 4. Hypoperfusion caused by hemorrhage, and CBF restoration by facial nerve stimulation in experimental group. Pig experiments, n=5 per group. Immediate post-hemorrhage (PH), 60 minutes post-hemorrhage (PH60), immediate post-stimulation (PS) and 60 minutes post-stimulation (PS60). P<0.05 by repeated-measures ANOVA.