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Selection of preclinical models to evaluate intranasal brain cooling for acute ischemic stroke

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Abstract:

Stroke accounts for a large proportion of global mortality and morbidity. Selective hypothermia, via intranasal cooling devices, is a promising intervention in acute ischemic stroke. However, prior to large clinical trials, preclinical studies in large animal models of ischemic stroke are needed to assess the efficacy, safety, and feasibility of intranasal cooling for selective hypothermia as a neuroprotective strategy. Here, we review the available scientific literature for evidence supporting selective hypothermia and make recommendations of a preclinical, large, animal-based, ischemic stroke model that has the greatest potential for evaluating intranasal cooling for selective hypothermia and neuroprotection. We conclude that among large animal models of focal ischemic stroke including pigs, sheep, dogs, and nonhuman primates (NHPs), cynomolgus macaques have nasal anatomy, nasal vasculature, neuroanatomy, and cerebrovasculature that are most similar to those of humans. Moreover, middle cerebral artery stroke in cynomolgus macaques produces functional and behavioral deficits that are quantifiable to a greater degree of precision and detail than those that can be revealed through available assessments for other large animals. These NHPs are also amenable to extensive neuroimaging studies as a means of monitoring stroke evolution and evaluating infarct size. Hence, we suggest that cynomolgus macaques are best suited to assess the safety and efficacy of intranasal selective hypothermia through an evaluation of hyperacute diffusion-weighted imaging and subsequent investigation of chronic functional recovery, prior to randomized clinical trials in humans.

Keywords:

Intranasal cooling, ischemic stroke, nonhuman primate, translational stroke research

Introduction

Ischemia during stroke leads to cellular injury and death through a series of biochemical reactions known as the ischemic cascade.[1-3] Neuroprotective therapies aim to interrupt the biochemical, cellular, and metabolic processes of injury during ischemia, and as such, could be effective treatments for ischemic stroke.[2] Despite numerous neuroprotective therapies being tested in animal models of ischemic stroke, none are currently approved for use in clinical practice.[4] The lack of efficacy of stroke therapies in clinical settings is thought to stem from the fact that most compounds only target a single step in the ischemic cascade and in specific cell types.[5] Therapeutic hypothermia is a promising neuroprotective therapy as it targets multiple steps in the ischemic cascade and mitigates injury by decreasing energy depletion, blood–brain barrier disruption, excitotoxicity, and apoptosis.[3,5-7] The potential of hypothermia as a neuroprotective therapy is underscored by successes in randomized clinical trials in patients with global cerebral ischemia after cardiac arrest and in neonates with hypoxic-ischemic encephalopathy.[8-10] However, to date, there are few clinical studies assessing the utility of hypothermia

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in ischemic stroke and no large-scale randomized clinical trials.[11-14]

Evaluation of Preclinical Evidence: is There Enough for Large Clinical Trials?

Preclinical studies in animal models of focal ischemia show promising results for hypothermia as a neuroprotectant. In a study of spontaneously hypertensive rats that underwent 90 min middle cerebral artery occlusion (MCAO), prolonged hypothermia (33°C for 24 h and then 35°C for 24 h) completely prevented contralateral limb deficits as revealed through a food retrieval task 1 month postinjury. Moreover, the authors found a significant reduction in infarct volume of rats that received hypothermia versus normothermic controls.[15] In another study where rats were subjected to 120 min of MCAO, animals that were exposed to whole body hypothermia of 33°C for 5 h showed a significant increase in survival rate and improvement in functional outcome as compared to normothermic controls. The authors also noted a significant decrease in the extent of hyperintensity in T2-weighted magnetic resonance imaging (MRI) scans and a reduction in cerebral edema in hypothermia-treated animals.[16] A 2007 systematic review and meta-analysis of 86 publications including data from 3,353 animals found that hypothermia reduced infarct size by 43.5% and improved functional outcome by 45.7%.[17]

Most preclinical studies on therapeutic hypothermia in ischemic stroke have been conducted in rodents; however, there are a few large animal studies including a feasibility and safety study conducted in baboons.[18-20] Using an endovascular heat transfer catheter, baboons underwent cooling to 32°C initiated 3 h after onset of ischemia (60 min MCAO) or were maintained in normothermic range. While not statistically significant, the authors noted that at 72 h, 75% of hypothermic animals (6 of 8) were able to feed themselves compared to only 38% of control animals (3 of 8). Moreover, the authors reported a 50% reduction in 72 h infarct volume of hypothermic versus normothermic animals (17 ± 8 and 32 ± 9% of ipsilateral hemisphere in hypothermic [$n = 6$] and normothermic [$n = 8$] animals, respectively). The authors did not note any differences in infarct size or neurobehavioral score at 10 days postinjury; however, given the low survival rate (control $n = 2$; hypothermia $n = 5$) to this time point, conclusions about efficacy must be drawn with caution.[20]

Translational research requires the use of numerous animal models depending on the focus of the study, which may differ significantly depending on the stage of therapy development.[21] As outlined in a 2010 review article, the process of stroke therapy development begins with (1) elucidating processes of cell injury and death and the discovery of potential therapeutic targets. The experimental requirements during this initial phase are model flexibility and access to a broad range of scientific tools. As such, murine models and human cell cultures are ideal. (2) Subsequently, there is a process of candidate therapy selection. At this stage, there is a need for the reliable detection of beneficial and harmful effects of specific therapies and the ability to rank candidates in terms of potency and efficacy. At this stage, the use of a more homogeneous population of rodents (inbred) to reduce variability could be beneficial. (3) Next, there should be preclinical verification of efficacy in the scope of circumstances that may be encountered in the clinic. As such, studies need to show a significant and persistent functional and physiological effect of the therapy in rodent models of comorbidities (aged, hypertension, diabetes, and atherosclerosis). (4) Finally, cross-species efficacy should be proven in a large animal (cats, pigs, sheep, and primates) model. Although the benefits are unproven, the Stroke Therapy Academic Industry Roundtable (STAIR) recognizes the distinct advantage of gyrencephalic nonhuman primates (NHPs) for this purpose because of their similarity to humans.[21,22]

Preclinical studies of therapeutic hypothermia fulfill the above first, second, and third requirements. (1) Studies in rodent models have revealed numerous molecular mechanisms of neuroprotection conferred by hypothermia. For example, cooling the ischemic rat brain to 33°C and 30°C completely inhibited the excitotoxic 7-fold increase in glutamate release and attenuated the 500-fold dopamine release by 60% in response to ischemic injury.[23] (2) There are numerous methods of hypothermia induction including surface, endovascular, and intranasal cooling. The efficacy of these different methods in their ability to cool the brain and possible adverse effects are well documented.[24-27] (3) The effect of therapeutic hypothermia has been tested in models of comorbidity including older, hypertensive, and diabetic animals, as well as in female animals. These studies showed no adverse effects of treatment in animals with comorbidities, and in fact, hypothermia was found to be slightly more effective in hypertensive rats than in normotensive animals.[17]

Finally, although there are feasibility and safety studies of therapeutic hypothermia in large animal models of stroke,[18-20] there are currently no published efficacy studies, rendering the last requirement of efficacy testing in multiple species before large-scale clinical trials unfulfilled. As such, the following proposed study of hypothermia in a large animal model of ischemic stroke could provide additional and valuable evidence to support or refute the therapeutic efficacy of hypothermia before a large-scale clinical trial.

Considerations of a Preclinical Large Animal Study Evaluating Hypothermia

Stroke model

Because middle cerebral artery (MCA) occlusion is the most common presentation of ischemic stroke in humans (occurring in 70% of strokes of vascular occlusion), most experimental models occlude the MCA.[28-30] As hypothermia is a neuroprotective therapy, the stroke model must exhibit an ischemic penumbra, and as such, the photothrombotic model will not be considered for this study.

One method of stroke induction that produces an ischemic penumbra is with the use of an intraluminal suture introduced through the internal carotid artery (ICA) and advanced until it interrupts the blood supply to the MCA.[21] Sutures closely mimic human ischemic stroke and allows reperfusion and the precise control of ischemia duration. However, this method can lead to subarachnoid bleeding and hemorrhaging and can sometimes result in inadequate occlusion and spontaneous recanalization depending on the type of suture, which could increase variability in infarct size.

Focal application of endothelin-1 (ET-1) is a vasoconstrictive peptide which can be applied directly to the MCA, through an intracerebral stereotactic injection, or onto the cortical surface and leads to a dose-dependent ischemic lesion with marginal ischemic edema.[21,31] This model can be minimally invasive and results in low mortality. The main drawbacks are that duration of ischemia is difficult to control, which results in low reproducibility, and the presence of ET-1 receptors on multiple cell types results in induction of astrocytosis and axonal sprouting.[32]

Embolic stroke models include the insertion of microspheres or thromboembolic clots into the MCA via the external carotid artery using a microcatheter.[21] This stroke model results in multifocal and variable infarcts. While it most closely mimics human strokes, it results in very low reproducibility of infarct location and high variability in infarct size and allows the possibility of spontaneous recanalization.

Finally, a craniotomy or craniectomy model allows direct surgical MCAO using electrocoagulation to create permanent occlusion or using microaneurysm clips, hooks, or ligatures to create transient occlusion.[21,30] The main advantage of this model is its reproducibility of infarct size and neurological deficits, low mortality, and direct visualization of successful MCAO and reperfusion (in transient models). While this technique affects intracranial pressure and blood–brain barrier function and requires significant surgical skill, with access to sufficient resources, the craniectomy model is ideal for preclinical large animal studies of ischemic stroke. Importantly, the use of this model of stroke induction with its high degree of reproducibility allows detection of statistically significant differences between treatment groups with smaller sample sizes, a crucial consideration for ethical and financial reasons.

Species selection

Functional outcome and behavioral assessments

Strokes in humans are characterized by sensorimotor and cognitive impairments, and animal models that mimic and allow assessment of such deficits are useful in evaluating the specific benefits of therapies. Several large animal models including pigs, sheep, dogs, and NHPs have been used in focal ischemic stroke studies each with a set of available neurobehavioral outcome measures. The open field test is the most commonly used assessment for evaluating locomotor activity and emotional reactivity in pigs. Previous studies have employed gait analyses to quantitatively assess deficits and found an asymmetric gait with reduced velocity following stroke.[33,34] Cognitive assessments include the novel object test, which can be used to assess behavioral responses to novelty.[35]

Ovine models of focal ischemic stroke are relatively new, and the few studies that have been conducted have used a qualitative stroke scale assessment detailing consciousness, ataxia, circling behavior, and compensatory motor behaviors such as hopping and hemistanding.[36]

Dog models of cerebral ischemia similarly use a scaled assessment to evaluate motor function, consciousness, head turning, circling, and hemianopsia.[37,38]

The battery of available neurobehavioral assessments in NHPs depends on the species, but is generally more numerous than tests available for other animals. Baboons are behaviorally aggressive, and assessments are limited to observational scoring on scales.[39] The NHP stroke scale (NHPSS) is a qualitative scaled assessment of consciousness, upper limb function, hemiparesis, visual field deficits, neglect, and spasticity.[30,39] Macaque monkeys are milder in temperament, and as such, a wider array of assessments can be employed following stroke. They can be trained to perform cognitive and motor tasks such as the 2-tube choice test, the hill and valley staircase where animals reach through vertical slots to retrieve food from a 5-step staircase, and the 6-well task tests where animals must use fine motor movements of the fingers to retrieve treats from 6 small wells within a plate. These tasks can reveal hand dominance and can be used to differentiate between hemiparesis and neglect by testing each arm in an isolated sensory field. Quantitative

measures of reaction time and movement time during task performance can provide additional information about sensory processing and motor control.[30,40,41] Common cognitive assessments of working memory include the delayed response test where animals are presented with an array of wells where a single well contains a food reward, and animals must remember the location of the reward after a delay. Studies of dementia have employed assessments of higher cognitive function including the delayed nonmatching to sample task, the delayed recognition span task, and the conceptual set shifting task.[40] In addition, the use of assistive robotic devices has become more widespread, and animals can be trained to perform visually guided reaching tasks and undergo assessments of spasticity and postural control while their arm positions are recorded over time to allow quantitative characterization of sensorimotor control.[42,43] The wide array of available assessments of motor, sensory, and cognitive deficits following stroke in NHPs together provide a very complete picture of numerous aspects of deficits that occur following stroke.

Cerebral vasculature and stroke induction considerations

Pigs have a gyrencephalic brain with a gray-to-white matter ratio similar to that of humans.[44] In pigs, as in humans, the ICA supplies the majority of blood to the cerebrum.[33] The intracranial ICA branches into two MCAs (one coursing laterally and the other more rostrally, compared to a single MCA in humans) and an anterior cerebral artery (ACA).[44] A pivotal study showed that electrocoagulation of the two MCAs results in consistent and reproducible infarcts and hemiparesis in both the forelimb and hindlimbs.[33,35] In contrast to humans, pigs have a rete mirabile, a network of small bilaterally connected vessels perfused by the ascending pharyngeal artery and from which the intracranial ICA originates.[33,35] This structure prevents direct communication (arteriolar diameter <1 mm) between the extra and intracranial segments of the ICA and is hypothesized to have evolved as a structure to help animals regulate brain temperature.[45,46] This anastomotic structure prevents embolization of large particles and the guiding of catheters, and as such, limits the available methods of stroke induction.

Ovine models of focal ischemic stroke have also been shown to be feasible, where MCAO studies have found histological changes consistent with ischemia.[36] The cerebrovascular anatomy of the sheep is also similar to that of a human's; intracranially, the ICA branches into the MCA and ACA, and the MCA supplies the majority of the motor and sensory areas of the lateral cortex. However, similar to the pig, sheep also have a rete mirabile from which the intracranial ICA originates.[47] The presence of this structure similarly prevents the implementation of embolic and suture methods of stroke induction.

Canines have also been used in previous preclinical studies of focal ischemic stroke. However, a major limitation of this model is the differences in cerebral vasculature of dogs compared to that of humans. For example, in humans, the internal carotid arteries are the main supply of blood to the brain via the Circle of Willis; however, in dogs, the cerebral arterial circle receives substantial blood supply from anastomotic vessels derived from the branches of the external carotid artery. The vertebral arteries of dogs are more important contributors of total blood supply to the brain than in humans.[48] Dogs also have a very rudimentary carotid rete. Ischemic stroke in the canine model has low reproducibility due to the extensive collateral circulation via the maxilla-carotid and meningocerebral anastomoses.[49] Even so, the major vessels supplying the cerebrum are same in humans and dogs, and MCA strokes in dogs induced through permanent occlusion or the introduction of autologous blood clots produce large cortical infarcts.[50]

Gyrencephalic NHPs such as macaque monkeys and baboons are phylogenetically closest to humans out of all the models considered above, and extrapolations of data from efficacy studies in NHPs may be better applied to clinical trials. The vascular anatomy of NHPs is analogous to that of humans. NHPs possess a complete Circle of Willis with most of their cerebral blood supply originating from the ICA. The ICA divides into an azygous ACA and MCA, which supply the midline and lateral aspects of the cerebrum, respectively.[21] Out of common NHP models, the cynomolgus macaque (*Macaca fascicularis*) may be most similar to humans in neurovascular anatomy in terms of the degree of collateralization; specifically, they have less collateral circulation than baboons and rhesus macaques (*Macaca mulatta*).[29,51] MCA strokes in NHPs affect sensory, motor, and cognitive function that are easily quantifiable.[30,40,41]

Nasal anatomy and vasculature

An additional necessary consideration given the selection of an intranasal cooling method of hypothermia induction and maintenance is the nasal anatomy and vasculature of potential animal models. Specifically, whether anatomy and vascular physiology constrain the feasibility or efficacy of intranasal cooling, and whether differences in these parameters may render results from a specific model less applicable to humans. The nasal cavity of an adult human is divided into two cavities (fossae) by the nasal septum; each fossa is the continuation of a nostril. On the lateral wall extending along the length of each cavity are three bones called conchae, also known as turbinates, which are responsible for forcing inhaled air to flow in a laminar pattern. The internal roof of the nasal cavity is composed of the perforated cribriform plate of the ethmoid bone through which sensory fibers of the olfactory nerve pass.[52,53]

The nasal structures in the animal models considered above are fairly similar to that of a human's with a few differences. The pig has a unique additional prenasal bone and changed lateral cartilages to support the tip of the nose and has relatively rigid nostrils.[54] The additional anatomical differences between the animal models are highlighted and compared in Table 1.

The vasculature supplying the nose is similar between dogs, sheep, pigs, NHPs, and humans. The sphenopalatine artery and its branches and the ethmoidal vessels are the two main groups of vessels that supply the nose. The sphenopalatine artery and its branches supply the respiratory portion of the nasal mucosa, and the ethmoidal vessels supply the olfactory region. In all the above-mentioned species, the sphenopalatine artery is a branch of the maxillary artery. In humans and NHPs, the ethmoidal group of arteries arises from the ophthalmic artery from the internal carotid; however, in the other species, they arise from a plexus in the olfactory fossa supplied by the external ethmoidal artery (a branch of the maxillary artery) and the internal ethmoidal artery (arises from the circle of Willis). The olfactory fossa plexus also receives supplementary vessels from a branch of the ACA, which supplies the olfactory bulb. In all species, the sphenopalatine artery enters the nose through the sphenopalatine foramen and branches into the nasopalatine artery that supplies the septum. Before reaching the maxillary turbinate, the sphenopalatine gives off several other branches to supply the turbinates, the posterior part of the lateral wall, and the maxillary sinus.[53,65]

In humans, the veins of the nasal cavity accompany the arteries. The anterior veins anastomose with the facial veins, and the posterior ethmoidal veins drain into the orbital and ophthalmic veins, pterygoid plexus, and cavernous sinus. Eventually, these veins drain into the external and internal jugular veins.[66,67] In sheep, pigs, and dogs, the posterior nasal veins also flow toward the cerebral vasculature draining into the sagittal sinus and infraorbital veins.[53] However, the cooler venous blood comes into close contact with the warmer arterial blood flowing through the rete.[68] NHPs, like humans, do not have a rete, and the nasal venous drainage does not interact with the cerebral arterial blood.[68]

Anatomical and vascular considerations for intranasal cooling

In artiodactyls (even-toed ungulates including sheep and pigs), arterial blood destined for the circle of Willis first travels through the vessels of the rete mirabile. The rete lies in a lake of venous blood in the cavernous sinus and is hypothesized to help animals regulate brain temperature during hot conditions and during exercise.[45,46] Indeed, in primates, the brain is always warmer than the arterial blood in the aorta or the common carotid. In contrast, sheep, dogs, and pigs have brain and cerebral artery temperatures that can fall below central arterial blood temperature, suggesting that heat exchange is occurring between warm arterial blood in the carotid rete and venous blood cooled by evaporation in the nasal cavity.[69] Given its postulated role in brain temperature regulation, the presence of a carotid rete in sheep, pigs, and to a lesser extent in dogs could pose challenges during the translation of selective hypothermia to humans because heat exchange and cooling could be occurring differently between these species. While not its main intended purpose, a feasibility study compared intranasal cooling in animals with (piglet) and without (rabbit) a carotid rete. Animals of comparable sizes (rabbits: 3.5 kg, piglets: 2.7 kg) underwent intranasal cooling using a vortex tube that introduced room temperature or −7°C air. Using room temperature air, the authors were able to achieve a rate of brain cooling of 3.7°C/h in rabbits, which was not significantly different from the 4.5°C/h rate achieved in piglets. Similarly, the use of −7°C air resulted in faster cooling rates that were not significantly different between the two species (rabbits: 5.2°C/h, piglets: 5.5°C/h). Importantly, even though piglets possess a carotid rete and rabbits do not, the rates of brain cooling using this intranasal device were not significantly different.[70]

However, a review of existing studies on intranasal cooling in adult sheep, pigs, and humans suggests that the rate of cooling that can be achieved may differ between these species [Table 2]. Specifically, brain cooling rates are faster in sheep and pigs than they are in humans, and this difference could be because of the presence of carotid rete, gross nasal anatomy differences, or a combination of the two. Therefore, since the presence of a carotid rete and anatomical differences could significantly affect the

Table 1: Anatomical parameters of nasal cavity of animal models considered compared to humans

Species	Distance from naso-pharynx to brain (cm)[55,56]	Cerebral blood flow (ml/100 g/min)[57-60]	Nasal volume (ml)[56]	Nasal surface area (cm^2)[59]	Brain size (cm^3)[61]	Body weight/brain weight ratio[18,62-64]
Human	2.3	50	20	160	1450	0.020/1
Pig	7.4	54	-	-	110	0.003/1
Dog	10.5	54	20	221	77	0.006/1
NHP	2.4	52	8	62	95	0.004/1
Sheep	9	55	114	327	93	0.002/1

NHP: Nonhuman primate

Table 2: Studies that have induced hypothermia using intranasal cooling in different species and the rates of cooling that were achieved

Study	Animal model	Baseline cerebral temperature (°C)	Cooling method used	Rate of cooling achieved (°C/h)	Target temperature (°C)
Chava *et al*., 2017[71]	Pig	36.8±1.2	Intranasal cold air		
			At 20 L/min	4.8	Not specified
			40 L/min	6.2	Not specified
			80 L/min	7.8	Not specified
Covaciu *et al*., 2008[18]	Pig	38.1±0.6	Nasal balloon catheter	8.4	35.0
Boller *et al*., 2010[72]	Pig	Not specified	RhinoChill	4.2	Not specified
Wolfson *et al*., 2008[19]	Sheep	Not specified	Intranasal perfluorohexane	13.8	Not specified
Poli *et al*., 2014[73]	Human	36.7±0.9	RhinoChill	1.2	34-35 (8 of 10 patients)
					36-36.5 (2 of 10 patients)
Covaciu *et al*., 2011[27]	Human	Not specified	Nasal balloon catheter	1.7	Not specified

efficacy of intranasal cooling, these and considerations of cerebrovascular physiology as it relates to stroke induction method and reproducibility, similarity to humans for ease of translation, and the availability of assessments of functional outcome must all be taken into account during the process of model selection. As such, NHP**s** such as the cynomolgus macaque are an ideal large animal model species in which to test the efficacy of therapeutic hypothermia in focal ischemic stroke.

Outcome measures

Behavioral assessments of sensory, motor, and cognitive deficits poststroke in cynomolgus macaques could include the NHPSS, 2-well task, hill and valley task, the delayed response task, and robotic assessments. Indeed, given the wide array of available assessment methods with the use of this animal model, as many tests and at as many time points as feasibly possible without inducing undue stress should be performed to help form a complete and comprehensive evaluation of deficits.

Cynomolgus macaques are also amenable to neuroimaging using MRI to determine infarct size, cerebral blood flow, anatomical and functional brain connectivity, and the rate of stroke evolution. T1- or T2-weighted anatomical imaging has been used extensively for characterization and quantification of infarct size in both preclinical animal models and human studies. Imaging sequences such as pseudo-continuous arterial spin labeling (pCASL) have been developed and optimized to allow quantification of cerebral blood flow, and time-of-flight MR angiography can be useful for evaluating intracranial circulation.[74,75] Diffusion tensor imaging (DTI) and resting state functional MRI (rs-fMRI) can be used to reconstruct white matter tracts through the brain and elucidate brain areas and networks that are functionally connected, respectively.[76,77] Of particular relevance to a study on the neuroprotective effects of therapeutic hypothermia is the ability to monitor stroke evolution by quantifying perfusion/diffusion mismatch over time. In acute stroke, a region that shows both diffusion and perfusion abnormalities is thought to represent irreversibly infarcted tissue, whereas a region that only shows perfusion abnormalities but has normal diffusion likely represents viable ischemic tissue (the penumbra) given timely reperfusion.[78] Therefore, if hypothermia confers neuroprotection through a slowing or halting of the ischemic cascade, and as such, ischemic damage, animals treated with hypothermia should show a slower rate of diffusion hyperintensity development than normothermic controls. As such, neuroimaging techniques can be used to determine whether treatment with therapeutic hypothermia can reduce infarct size and rate of stroke evolution and help better maintain cerebral blood flow, white matter tract integrity, and functional connectivity.

Postmortem, outcome measures could include histological verification of infarct size using hematoxylin and eosin (H and E) at a chronic time point or quantification of amount of metabolically inactive tissue using tetrazolium chloride (TTC) at an acute time point.[79,80]

Proposed Studies

Prior to a large-scale randomized clinical trial, we recommend the following two preclinical studies on the efficacy of therapeutic hypothermia in acute ischemic stroke: an acute terminal study and a chronic recovery study. As per the STAIR recommendations, any proven efficacy should be independently replicated in a separate laboratory. Moreover, both studies should include adequate randomization to treatment groups (hypothermia versus normothermic controls), allocation concealment, blinded outcome assessment, and rigorous control physiologic parameters (blood pressure, heart rate, respiratory rate, core temperature, and brain temperature either using probes directly implanted in the brain or tympanic temperature as a proxy) for the duration of the procedure.

Preliminary acute study

In a preliminary acute study, strokes will be induced in cynomolgus macaques using a craniectomy model of permanent MCAO with electrocoagulation. The primary outcome of this initial study will be the rate of stroke evolution as measured through perfusion/diffusion mismatch. The secondary outcome measure will be postmortem histological quantification of amount of metabolically inactive tissue using TTC staining. Immediately after stroke induction, animals will be transferred to an MRI for perfusion and diffusion imaging. Animals will then be randomized to a treatment group, and hypothermia treatment or normothermic maintenance will begin an hour after ischemia onset. After treatment initiation, animals will be serially imaged once an hour for 6 h, after which time, they will be sacrificed, and their brains collected and stained using TTC.

Electrocoagulation of the MCA will ensure that there will not be a distortion on perfusion and diffusion images (such as from a nonferromagnetic aneurysm clip). A permanent model of MCAO without reperfusion is selected for this initial study, so that there is continuous stroke evolution, the rate of which can be measured across the 6-h study duration. Finally, a 6-h termination point is selected due to ethical considerations, and previous studies have shown that with this model of permanent occlusion in cynomolgus macaques, stroke evolution is complete by this time point. We would recommend proceeding to the following proposed chronic recovery study only if hypothermia shows a decrease in the rate of stroke evolution in this initial acute study.

Chronic recovery study

In the chronic recovery study, animals will first undergo baseline behavioral assessment and neuroimaging (T1, T2, diffusion-weighted imaging, pCASL, rs-fMRI, and DTI). Strokes will then be induced in these animals using a craniectomy model of transient MCAO with a nonferromagnetic aneurysm clip. A transient model of MCAO more closely mimics clinical conditions because of treatment of human patients with rtPA and high rates of spontaneous recanalization. Animals will be randomized to a treatment group 60 min after stroke onset, and reperfusion will occur after 90 min of ischemia. They will undergo the same neuroimaging protocol as described above for the first 6 h of treatment. After 6 h, animals will be slowly rewarmed and recovered.

Follow-up behavioral assessments will occur at 8 h postrecovery and daily thereafter. Follow-up neuroimaging will occur at 48 h, 1 week, and 1-month poststroke of the same sequences as prestroke baseline for comparison. Animals will undergo their final behavioral and neuroimaging assessments at 1 month poststroke, after which point, animals will be terminated, and their brains collected, fixed, and processed for H and E staining. A termination date of 1 month poststroke is selected to determine whether treatment efficacy is persistent.

Other considerations

Even given proven efficacy of therapeutic hypothermia in a large animal model of ischemic stroke, there are a few additional considerations before a large clinical trial. Specifically, the pharmacokinetic efficacy of compounds commonly prescribed to individuals at high risk of ischemic stroke (e.g., hypertension and diabetes) must be evaluated in conjunction with hypothermia to ensure no adverse effects, such as with the use of rodent models. Careful considerations of serious adverse effects observed in the above-proposed animal studies (if any) should also be addressed.

Conclusions

There is ample evidence from small animal preclinical studies to support the use of therapeutic hypothermia in ischemic stroke; however, before a large-scale randomized clinical trial in acute ischemic stroke patients, the efficacy of hypothermia induced through intranasal cooling must be verified in a large animal model of stroke. We propose the use of cynomolgus macaques with strokes induced through MCAO to assess the efficacy of said therapy as revealed through neuroimaging and neurobehavioral assessments of sensory, motor, and cognitive deficits.

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Conflicts of interest

There are no conflicts of interest.

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