URL: https://stroke-submit.aha-journals.org/

Manuscript Number: STROKE/2021/036050R1

Title: Comparison of Large Animal Models for Acute Ischemic Stroke: which model to use?

Authors:
Aladdin Taha (Erasmus Medical Center)
Joaquim Bobi (Erasmus MC)
Ruben Dammers (Erasmus Medical Center)
Rick Dijkhuizen (University Medical Center Utrecht)
Antje Dreyer (Max Planck Institute for Infection Biology)
Adriaan van Es (Leiden university Medical Centre)
Fabienne Ferrara (Fraunhofer Institute for Cell Therapy and Immunology (IZI))
Matthew Gounis (University of Massachusetts Medical School)
Bjoern Nitzsche (University Leipzig)
Simon Platt (University of Georgia)
Michael Stoffel (University of Bern)
Victor Volovici (Erasmus MC)
Gregory del Zoppo (University of Washington)
Dirk Duncker (Erasmus MC, University Medical Center Rotterdam)
Diederik Dippel (Erasmus University Medical Center)
Johannes Boltze (The University of Warwick)
Heleen van Beusekom (Thoraxcenter, Erasmus University Medical Center)

Comparison of Large Animal Models for Acute Ischemic Stroke: which model to use?

Aladdin Taha, MD[1,2]*; Joaquim Bobi, DVM, PhD[1]*; Ruben Dammers, MD, PhD[3]; Rick Dijkhuizen, PhD[4]; Antje Y. Dreyer, DVM, PhD[5]; Adriaan van Es, MD, PhD[6]; Fabienne Ferrara, DVM, PhD[7]; Matthew J. Gounis, PhD[8]; Björn Nitzsche, DVM, PhD[9,10]; Simon Platt, BVM&S, FRCVS[11]; Michael H. Stoffel, DVM, PhD[12]; Victor Volovici, MD, PhD[3]; Gregory J. del Zoppo, MD, MS[13]; Dirk J. Duncker, MD, PhD[1]; Diederik W.J. Dippel, MD, PhD[2]; Johannes Boltze, MD, PhD[14]†; Heleen van Beusekom, PhD[1]†.

1. Division of Experimental Cardiology, Department of Cardiology, Erasmus MC University Medical Center, Rotterdam, the Netherlands; 2. Erasmus MC University Medical Center, Stroke Center, Department of Neurology, Rotterdam, the Netherlands; 3. Erasmus MC University Medical Center, Stroke Center, Department of Neurosurgery, Rotterdam, the Netherlands; 4. Biomedical MR Imaging and Spectroscopy Group, Center for Image Sciences, University Medical Center Utrecht, Utrecht University, Utrecht, the Netherlands; 5. Max Planck Institute for Infection Biology, Campus Charité Mitte, Berlin, Germany; 6. Department of Radiology, Leiden University Medical Center, Leiden, the Netherlands; 7. Fraunhofer Institute for Cell Therapy and Immunology (IZI), Leipzig, Germany; 8. Department of Radiology, New England Center for Stroke Research, University of Massachusetts Medical School, Worcester, MA, USA; 9. Institute of Anatomy, Faculty of Veterinary Medicine, University of Leipzig, Leipzig, Germany; 10. Department of Nuclear Medicine, University of Leipzig, Leipzig, Germany; 11. Department of Small Animal Medicine and Surgery, College of Veterinary Medicine, University of Georgia, Athens, GA, USA; 12. Division of Veterinary Anatomy, Vetsuisse Faculty, University of Bern, Bern, Switzerland; 13. Division of Hematology, Department of Medicine, Department of Neurology, University of Washington School of Medicine, Seattle, WA, USA; 14. School of Life Sciences, University of Warwick, Coventry, UK.

* These authors contributed equally to this study.

† These senior authors contributed equally to this study.

Correspondence to: Heleen M.M. van Beusekom, Erasmus MC, University Medical Center Rotterdam, Division of Experimental Cardiology, Department of Cardiology, Office Ee2393a, Wytemaweg 80, Rotterdam, 3015CN, the Netherlands. E-mail: h.vanbeusekom@erasmusmc.nl; Tel: +31(0)107044048.

Article type: Review

Cover title: Large Animal Models for Acute Ischemic Stroke

Tables and figures: Tables 2; Figures 3

Keywords: 'Stroke', 'Brain Ischemia', 'Animal Model', 'Dogs', 'Swine', 'Sheep', 'Non-Human Primates'

Subject terms: 'Animal Models of Human Disease', 'Cerebrovascular Disease/Stroke', 'Ischemic Stroke'

Total word count: 8345

Abstract

Translation of acute ischemic stroke (AIS) research to the clinical setting remains limited over the last few decades with only one drug, recombinant tissue plasminogen activator, successfully completing the path from experimental study to clinical practice. To improve the selection of experimental treatments before testing in clinical studies, the use of large gyrencephalic animal models of AIS has been recommended. Currently, these models include, among others, dogs, swine, sheep and non-human primates, that closely emulate aspects of the human setting of brain ischemia and reperfusion. Species-specific characteristics, such as the cerebrovascular architecture or pathophysiology of thrombotic/ischemic processes, significantly influence the suitability of a model to address specific research questions. In this article, we review key characteristics of the main large animal models used in translational studies of AIS, regarding (*i*) anatomy and physiology of the cerebral vasculature, including brain morphology, coagulation characteristics and immune function, (*ii*) ischemic stroke modelling, including vessel occlusion approaches, reproducibility of infarct size, procedural complications and functional outcome assessment, and (*iii*) implementation aspects, including ethics, logistics and costs. This review specifically aims to facilitate the selection of the appropriate large animal model for studies on AIS, based on specific research questions and large animal model characteristics.

Non-standard Abbreviations and Acronyms

AIS	Acute Ischemic Stroke
CoW	Circle of Willis
ICA	Internal Carotid Artery
MCA	Middle Cerebral Artery
MCAO	Middle Cerebral Artery Occlusion
NHP	Non-Human Primates
RM	Rete Mirabile
rt-PA	Recombinant tissue Plasminogen Activator

Introduction

Animal models of acute ischemic stroke (AIS) are instrumental to translate novel experimental therapies from the laboratory to the clinical setting. In 1999 and again in 2009, the Stroke Therapy Academic Industry Roundtable (STAIR) recommended the use of large gyrencephalic models as an important translational step together with rodent studies as a complement to clinical trials.[1,2] AIS models have been developed in several species including dogs, swine, sheep and non-human primates (NHP). A selection between these models is relevant when translating basic science research to clinical studies.

Rodent models are invaluable for experimental stroke research due to the availability of transgenic and immuno-compromised strains, ethical acceptance, and low costs. However, with the exception of recombinant tissue plasminogen activator (rt-PA), to date no drugs have been proven effective in AIS patients despite numerous successful drug studies in rodents.[3] This poor translatability has been linked to preclinical as well as clinical methodological/trial design issues, e.g. sensitivity to detect modest effects and failure to mirror study design.[4,5] However, this could also, at least partially, be explained by anatomical differences between the human and rodent brain with respect to size, morphology (gyrencephalic versus lissencephalic), and gray/white matter ratio.[6] Consequently, the STAIR consortium has recommended the addition of a large gyrencephalic model as a critical extra step in the translational pathway towards clinical application.[1,2]

Large animal models enable application of the same clinical diagnostic imaging modalities and therapeutic devices as in humans. Use of the same scanners and scanning protocols as for human patients facilitates translation of research findings. Another advantage is the larger circulating blood volume, as compared to rodents, which allows for extensive and safe serial blood sampling (**Supplemental Material**) to study, for instance, kinetics of plasma biomarkers of ischemia or pharmacokinetics of novel therapeutics. Large animal models are

particularly valuable in areas of translational stroke research, investigating novel devices that require a larger brain or larger diameter of cerebral vessels. For instance, a recent study in dogs showed that catheter-based focal brain cooling initiated prior to recanalization was safe and reduced infarct size as compared to recanalization alone.[7,8] Clearly, this type of catheter-based intervention could not have been performed in a rodent model. Similarly, responses to currently employed clinical interventions such as mechanical thrombectomy should ideally be studied in large animal models.[9] Moreover, large animal models can be useful to explore opportunities emerging from sophisticated imaging approaches in stroke[10] and related fields.[11]

In order to improve the quality of pre-clinical research, the Planning Research and Experimental Procedures on Animals Recommendations for Excellence (PREPARE) guidelines were introduced in 2018 and point to the "assessment of the relevance of the species to be used, its biology and suitability to answer the experimental questions with the least suffering, and its welfare needs."[12]

In light of these considerations, the aim of this review is to guide AIS investigators in choosing the appropriate large gyrencephalic animal model to address their specific research question. This review originated from our quest to set up our own large-animal model of AIS. We wanted to address issues that were raised discussions with leading experts on large-animal models of AIS about how to address the most relevant questions when establishing a large-animal model of AIS. For this purpose, we reviewed the literature and compared important features of dog, swine, sheep and NHP models. Cats also have a gyrenchephalic brain and were among the first large animals being used in stroke research.[13-15] Despite their pioneering role, cat stroke models are nowadays less frequently used, potentially due to ethical considerations and less demanding husbandry of similarly-sized (lissencephalic) rabbits. Therefore, cat stroke models are not included in this review.

Here, we discuss *(i)* comparative anatomy and physiology, including cerebral vasculature, brain morphology, and coagulation characteristics and immune function, (*ii*) ischemic stroke modelling, including vessel occlusion approaches, reproducibility of infarct size, procedural complications and functional outcome assessment, and (*iii*) implementation aspects, including ethical aspects and practical aspects, such as logistics and costs. Taking this approach, this review aims to provide a rational and informed approach for investigators to select the most appropriate large animal model for their particular research.

i. Comparative anatomy and physiology

Structure and function of the cerebral vasculature

Lesion size and reproducibility of the ischemic injury are key determinants of a stroke model's translational value. Both factors largely depend on cerebrovascular anatomy and variability. The middle cerebral artery (MCA) provides the major proportion of blood volume to the brain, particularly to motor and sensory areas. It is the most common occlusion site in human AIS, making it the most important site to induce focal cerebral ischemia in animal models.

In humans, large artery occlusion mostly occurs in M1 (main stem) or M2 segments of the MCA.[16] Proximal MCA occlusions in humans, dogs, swine, sheep, and NHP have all shown to affect the ipsilateral cortex and, when the occlusion is located proximal to the lenticulostriate arteries, the basal nuclei as well.[17-21] The origin (terminal branch of the internal carotid artery [ICA]) and branching of the MCA are similar in humans, dogs and NHPs. In swine and sheep, the MCA branches from the circle of Willis (CoW) and these animals sometimes have a duplicate or even triplicate MCA.[22]

The MCA diameter is generally smaller in animals as compared to humans. In humans, the diameter is 3.2±0.3 mm,[23] whereas the reported external diameters are 1.5±0.3

mm in dogs, 1.3±0.3 mm in sheep, and 1.2±0.1 mm in macaques (*Macaca mulatta*) (mean ± SD).[24] In swine, MCA diameter is 1.2 mm,[25] whereas in baboons the diameter is up to 2.0 mm (personal communication GDZ). Vessel sizes are also strain- and age-dependent. The vessel diameters are relevant for development of catheter-based methods using similar catheters as in the human setting. Nonetheless, the capacity to reach the MCA endovascularly also depends on the anatomical configurations of the head-neck vasculature, namely the access to the CoW and the existence of a physiological *rete mirabile epidurale rostrale* (RM). This is an anatomical structure composed of a compact and complex network of anastomosing vessels, interlaced with a venous plexus located in the cavernous sinus (**Figure 1**), and has been used to model vascular malformations as seen in humans.[26] It is unclear whether the RM affects distal blood pressure and flow during recanalization.

Figure 2 provides a schematic overview of the CoW, and proximal and distal arteries of importance for each model. Humans, dogs and NHP do not have a physiological RM. Swine and sheep possess a RM located proximal to the intracranial ICA. The RM supplies a short ICA, which continues into the CoW. In sheep, the extracranial part of the ICA is obliterated after birth, and the RM is then fed by the maxillary arteries. In swine, the RM is fed by the ascending pharyngeal artery. The function of the RM is not fully understood, however, it effectively prevents the occurrence of thromboembolic stroke.[27] It also poses a limitation to the use of intravascular techniques for induction of focal ischemia in these species, as most catheters cannot pass the RM.[27] Catheter-based occlusions are feasible in dog and NHP. Moreover, the size of their brain vasculature allows for the use of microcatheters, stent-retrievers or coils used in routine (human) interventional radiology procedures.

The human MCA can react to stimuli such as hypercapnia by vasodilation,[23] and it is known that cerebral vasoregulation is impaired in stroke.[28,29] In baboons, the arterial supply and regional flow characteristics have been studied in the setting of normoxia and

ischemia.[30,31] However, while vascular reactivity of the coronary circulation following ischemia-reperfusion has been studied extensively in swine and dogs,[32,33] studies on cerebrovascular reactivity in both species are scarce. In swine, cerebral traumatic injury was shown to influence vascular reactivity to acetylcholine and hypocapnia. This change in reactivity depends on injury size.[34] Knowledge of the vascular response to ischemia in the various large animal models will add to the interpretation of research findings.

Brain structure and gray and white matter

The translational value of a stroke model also depends on the respective species' brain morphology, weight, and complexity. Humans, dogs, swine, sheep and NHPs are all gyrencephalic species and have a higher percentage of white matter compared to lissencephalic animals such as rodents. The percentage of white matter also increases with brain size.[35] Gray and white matter have different metabolic demands as their vulnerability to ischemia and collateral blood supply differ.[36,37] Literature indicates intra-species variations in white matter percentages, but this depends on whether the white matter is given as a percentage of the cortex or of the total brain. Percentages of white matter in the cortex of large animals (swine 28.4%; sheep 27.7%; NHP 27.7% (*Macaca Mulatta*))[38] are closer to humans (40-45%) than that of rodents (10-12%).[6] Apart from susceptibility to ischemia, different percentages and organization of white matter are relevant when studying brain connectivity (e.g. by MRI diffusion tensor imaging). Compositional differences between non-human primates (e.g. Papio *sp*, Macaca *sp*) and rodents (e.g. locations and distributions of organized white matter bundles in subcortex and basal nuclei) support the use of the former for human-relevant focal ischemia studies. This may (partially) explain failures of rodent interventional studies to translate to successful human treatments.[39] For instance, subcortical white matter typically represents a larger proportion of tissue at risk following MCA

occlusion in larger primates than in rodents. In fact, in all large-animals described in this review, white matter is organized as the internal capsule, but in rats and mice many small fiber tracts are scattered throughout the basal ganglia gray matter.

Coagulation and immune function

In addition to ischemia as a result of the primary thromboembolic occlusion, AIS is known to induce thrombo-inflammation, an interaction between platelets and inflammatory cells, that can contribute to infarct maturation and growth.[40] Moreover, stroke triggers substantial central and peripheral immune reactions.[41] Therefore, it is important to consider the significant differences in the coagulation and immune function in the various large animal models as compared to humans. It is not clear whether these differences, e.g. shorter PT/PTT in dogs, have a direct effect on infarct size. However, they are particularly relevant when coagulation values and inflammation characteristics are measured as outcomes. A detailed description of relevant coagulation and immune system characteristics of dog, swine, sheep and NHP can be found in **Supplemental Material** and **Supplementary Table I**. Importantly, surgical procedures for the induction of stroke will influence and contribute to thrombo-inflammation, making sham animals essential for assessing coagulation and inflammatory responses triggered by the intervention.

ii. Ischemic stroke modeling

MCA occlusion (MCAO) in large animal models can either be performed endovascularly using a catheter-based approach, or externally using a neurosurgical approach, depending on the chosen species. **Table 1** summarizes the approaches for AIS models in dogs, swine, sheep and NHP as reported in previous studies. A more complete listing of NHP models, both in awake and anesthetized animals, is given in **Supplementary Table IV**. Neurosurgical and

endovascular methods can be used for permanent and transient MCAO. In transient occlusion models, occlusion times generally vary between one and four hours, similar to occlusion durations in AIS patients receiving reperfusion treatments. Duration of reperfusion is an important parameter to take into account. This is determined by the research question, for example acute experiments for mechanistic studies or long-term follow-up studies when assessing functional outcome.

External occlusion

External occlusion is achieved by specific neurosurgical approaches and can be used for both permanent (i.e. ligation, external compression or electrocoagulation) or transient (i.e. clipping, external compression or arterial thrombosis) occlusions. The use of removable microvascular clips allows for recanalization of the artery at a desired occlusion site and at a well-controlled point in time. Arterial thrombosis can be induced by arterial crush injury, chemical injury, or photothrombosis, which all tend to lead to platelet-rich occlusions, and recanalization can be achieved by thrombolysis.[42-45] Alternatively, severe vasoconstrictors such as endothelin-1 can be introduced intra-parenchymally to induce ischemia.[46] These options are not well-controlled in terms of the timepoint of reperfusion.

Of note, a craniotomy/craniectomy can be used for direct measurements on the brain tissue, including tissue oxygenation, microvascular function and flow, and functional monitoring using electroencephalography. Although the neurosurgical approach has benefits, including direct visualization of brain vasculature, it has also been associated with untoward effects that may influence outcome. Those comprise accidental brain tissue damage, hemorrhage, and altered intracranial pressure due to removal of the overlying bone, dural resection and loss of cerebrospinal fluid. The exposure of the MCA may require enucleation in some approaches, as performed in an AIS model in baboons (*Papio species*), using an

external balloon occlusion.[47,48] Although neurological evaluation has been extensively performed in this model, loss of in-depth vision should be taken into consideration when designing behavioral assessment in follow-up experiments.

Endovascular occlusion

Endovascular models have been used for both permanent and transient MCAO. This approach is only possible in dogs and NHP, due to the RM in swine and sheep.

Different approaches have been developed and are used to induce endovascular MCAO in dogs and NHP, for example using thrombo-emboli, silicone plugs, coils, balloon catheters, nylon threads or micro-beads.[18,49-54] All of these are used for recanalization in AIS models, although when using micro-beads, there are limitations regarding specific occlusion of the MCA and recanalization. Of these methods, the thrombo-embolic occlusion most closely mimicks human stroke pathogenesis. In this type of model, an externally generated thrombus is injected using a guiding catheter selectively positioned in the targeted vasculature. Although this method closely emulates the pathogenesis of a large proportion of strokes in humans, it is less controllable than external occlusion and results in variable occlusion patterns across experiments. Thrombi can be created using autologous blood under static or flow conditions, which has a significant influence on thrombus composition and mechanical characteristics.[50,55,56] To circumvent the RM in swine, an endovascular model has been developed by injecting thrombin proximal to the RM.[53] This approach occludes most of the downstream vasculature causing massive strokes, but is less controllable than a targeted occlusion in dogs and NHP.

In order to study mechanical thrombectomy, swine extracranial arteries are often used for endovascular thromboembolic occlusion.[8,57,58] These models do not induce ischemic stroke, but are suitable for studying the success rate and associated endovascular damage of

recanalization strategies, and can effectively replicate the tortuosity of human brain-supplying vessels. Similar studies have been performed in dogs.[58]

Reproducibility of infarct size and procedural complication rate

Large animal studies, particularly for NHPs, require well-trained multidisciplinary research teams and specialized animal facilities for housing and for surgery.[59] Both from an ethical as well as an economics point of view, the number of studied animals should always be minimized by reducing variation between animals and standardizing research procedures. For these reasons, reproducibility of infarct size and location should be taken into account when deciding which animal model to use. In general, thromboembolic endovascular models inherently have a more variable occlusion site due to varying vascular anatomy. Additionally, clot fragmentation can cause secondary lesions, further adding to the variability of infarct size and location. External occlusion models tend to result in highly reproducible infarcts.[60,61] However, some species including dogs exhibit an extensive collateral circulation via maxilla-carotid and meningocerebral anastomoses, and a large draining cerebral vein that can reverse flow, which reduces reproducibility of infarcts and thus increases required sample sizes.[62] Infarct size in endovascular NHP models can also be highly variable.

Periprocedural complications, e.g. hemorrhages, also increase the number of animals required. Loss due to complications in the surgical approach in swine and sheep is typically 5 to 10%, including follow-up drop-outs due to uncal herniation as a result of edema, or epilepsy. Estimated complication rates for endovascular models in dogs tend to be higher,[51] mostly due to perforation of the highly tortuous arteries encountered for access to the MCA. However, complication rates for all species can significantly differ depending on, for example, the model of MCAO, occlusion duration, or center expertise.

Functional testing

Testing of functional outcome is not as widely applied in large animal models as in rodent models, in part due to study constraints (such as the surgical approach) and ethical considerations.[20] While behavioral assessments only require a baseline and follow-up measurement, assessment of cognitive function following stroke in large animals generally requires pre-training of the animals.[21,63,64] Given the gyrencephalic nature, large brain size and proportion of white matter, such tests can provide a closer model of functional outcome in humans as compared to rodent models.

Different neurological assessments have been described for different species. In dogs, various neurological scores are used to assess a combination of the following features: consciousness, motor function, sensory function, head position, gaze, hemianopia and circling.[18,51,65] A study in sheep added facial paralysis and ataxia outcomes.[60] Sensory function is difficult to test in sheep, as they habituate quickly to nociceptive stimuli.[60] In addition to the functional outcome measures mentioned above, swine AIS models also focused on appetite, vocalization[66], and standardized qualitative gait assessments using video recordings.[19,67] Analyzed parameters in gait assessment include swing time, stance time, step length, step velocity and hoof height.[67] Gait assessment methods developed in swine are less subjective and available for comparison. For NHP models, the Non-Human Primate Stroke Scale (NHPSS) was developed in order to standardize functional testing in an experimental stroke setting based upon similar experience in canine studies.[68] This scale is similar to the National Institutes of Health Stroke Scale (NIHSS) for humans. It studies the level of awareness, the ability to self-care, tone and posture, distal strength and coordination. Furthermore, cognitive tests such as the 6-tube search task are also used in NHP stroke models,[21] but not implemented in baboons. Although cognitive tests are also available for dogs[69], swine[70], and sheep[71,72] they are not yet widely applied in AIS models.

Various functional outcome assessment scales are available. However, following the NHPSS, a consensus on standardized and translatable scales for dogs, swine and sheep should be introduced to improve comparability between large animal stroke studies and translation to clinic.

iii. Implementation aspects

Ethical and societal considerations

In addition to anatomical and scientific aspects, in time some concerns over the management of animals both environmental and during and after experiments have come to influence the choice of specific large animal models. In the last two decades, there have been multi-level discussions about how to manage potential issues, however, in general, their solution has been a local and/or national affair. Legislation or local rules can restrict the use of specific species based upon experiment requirements, local housing and care capabilities, expertise, former experience, and other features felt to be important to the welfare of the animal (and researcher). In some instances national requirements/rules may only allow certain experiments when those studies are not feasible in other animals. For example, *de novo* setting up of dog and NHP biomedical research facilities is highly restricted in Europe.

Practical aspects

Another factor in determining the choice of the large animal model for AIS studies relates to the expertise available in the research center, as setting up a new animal model without existing infrastructure requires considerably more time and effort than rodent models and may carry considerable risk for the animal. For housing, dogs must have a courtyard for outside running and NHP should be able to run, climb and jump. Aside from this standard care, performing transcranial surgery or endovascular occlusions requires experience in

neurosurgery or neuro-interventions, respectively. This, together with experience in anesthesia and analgesia for the specific species, will reduce complication rates considerably and reduce the number of animals needed for setting up and optimizing the model.

Moreover, differences in costs should be considered and can vary per study site. NHP models are most similar to human patients but are also most expensive to acquire. Estimated costs for the four species are given in **Supplementary Table III**, but depend strongly on strain, age and comorbidity. Housing costs also vary per location, but tend to be higher for dog and NHP.

Selecting the most appropriate large-animal AIS model

The suitability of an animal model to answer a specific research question depends on the strengths of the model (over other available models) and on the associated practical challenges (**Table 2**). Therefore, different research settings (line of investigation, availability of dedicated facilities or specific expertise) might favor a different large-animal model. In other words, the most appropriate animal model for researcher A might not be the same as for researcher B. We have designed a flowchart (**Figure 3**) to assist in choosing an appropriate large animal model based on research questions and animal model characteristics.

The first aspect to be taken into account is the pathophysiological or therapeutic entity (perfusion studies, coagulation/thrombosis, (neuro-)immunology, neuroprotection or recanalization/thrombolysis) to be studied, and the main aim of the study (descriptive/proof of concept or to confirm robust results obtained in other animal models). Although thrombolysis in swine is closer to humans, and dogs and NHP are the most recognized models for confirmatory preclinical studies before moving into the clinical setting, there is no gold-standard model to study these topics. More elements have to be considered to properly select the animal model that suits your research and infrastructure.

The second and probably most important determinants are cerebrovascular anatomy and physiology. Dogs and NHP models are the first choice when using endovascular and autologous thromboembolism to induce AIS. However, this requires a skilled experimenter as ICA tortuosity can complicate the endovascular approach and increases the peri-procedural complications due to the risk of perforations and intracerebral hemorrhage. If AIS is not a prerequisite to study endovascular techniques, then extracerebral vessels, particularly in swine, are an excellent alternative.

When direct endovascular access to the brain is not an essential condition, the surgical AIS models in sheep and swine should be considered as they have lower associated costs, less housing requirements and less societal resistance. Moreover, the transcranial approach allows for precise control of occlusion site and occlusion duration. This enhances reproducibility of infarct size and location, and reduces the number of animals needed to achieve sufficient statistical power. The accurate timing of recanalization is especially useful when studying reperfusion. Trepanation also enables direct on-tissue measurements of the infarct zone, e.g. tissue oxygenation, microvascular function and flow, and functional monitoring using electroencephalography. Nonetheless, the different variants of the craniotomy to expose the MCA demand experienced staff. We recommend involving neurosurgeons and veterinarians to perform or assist in the surgery and manage or assist in anesthesia and post-operative care.

Conclusion

The decision of which animal model to use depends on the research question and available expertise and infrastructure. The dog and NHP model are suitable for endovascular approaches but their availability is limited, mainly due to ethical considerations and societal aspects. While the NHP model is considered the best approximation of the human situation, it requires highly specialized expertise and can be very costly. For the external surgical

approach, swine and sheep models are very suitable as they have lower associated costs, are easily available, and lead to less societal resistance.

Acknowledgements

Sources of Funding

We acknowledge the support of the Netherlands Cardiovascular Research Initiative which is supported by the Dutch Heart Foundation (CVON2015-01: CONTRAST), the support of the Brain Foundation Netherlands (HA2015.01.06), and the support of Health~Holland, Top Sector Life Sciences & Health (LSHM17016), Medtronic, Cerenovus and Stryker European Operations BV. The collaboration project is additionally financed by the Ministry of Economic Affairs by means of the PPP Allowance made available by the Top Sector Life Sciences & Health to stimulate public-private partnerships.

Disclosures

Matthew Gounis has been a consultant on a fee-per-hour basis for Astrocyte Pharmaceuticals, Cerenovous, Imperative Care, Medtronic Neurovascular, Mivi Neurosciences, Phenox, Q'Apel, Route 92 Medical, Stryker Neurovascular, and Wallaby Medical; holds stock in Galaxy Medical, Imperative Care, InNeuroCo and Neurogami; and has received research support from National Institutes of Health (NIH), the United States – Israel Binational Science Foundation, Anaconda, ApicBio, Arsenal Medical, Axovant, Cerenovus, Ceretrieve, Cook Medical, Galaxy Therapeutics, Gentuity, Imperative Care, InNeuroCo, Insera, Magneto, Microvention, Medtronic Neurovascular, MIVI Neurosciences, Naglreiter MDDO, Neurogami, Omniox, Philips Healthcare, Rapid Medical, Route 92 Medical, Stryker Neurovascular, Syntheon, ThrombX Medical and the Wyss Institute.

Diederik Dippel reports funding from the Dutch Heart Foundation, Brain Foundation Netherlands, The Netherlands Organisation for Health Research and Development, Health Holland Top Sector Life Sciences & Health, and unrestricted grants from Penumbra Inc., Stryker European Operations BV, Medtronic, Thrombolytic Science, LLC and Cerenovus for research, all paid to institution.

Johannes Boltze has been a consultant for Astrocyte Pharmaceuticals.

Supplemental Material

Online Tables I–IV

References 59,63,73-88

References

1. Fisher M, Feuerstein G, Howells DW, Hurn PD, Kent TA, Savitz SI, et al. Update of the stroke therapy academic industry roundtable preclinical recommendations. *Stroke*. 2009;40:2244-2250
2. Stroke Therapy Academic Industry R. Recommendations for standards regarding preclinical neuroprotective and restorative drug development. *Stroke*. 1999;30:2752-2758
3. O'Collins VE, Macleod MR, Donnan GA, Horky LL, van der Worp BH, Howells DW. 1,026 experimental treatments in acute stroke. *Ann Neurol*. 2006;59:467-477
4. Crossley NA, Sena E, Goehler J, Horn J, van der Worp B, Bath PM, et al. Empirical evidence of bias in the design of experimental stroke studies: A metaepidemiologic approach. *Stroke*. 2008;39:929-934
5. Stroke Therapy Academic Industry R, II. Recommendations for clinical trial evaluation of acute stroke therapies. *Stroke*. 2001;32:1598-1606
6. Boltze J, Nitzsche F, Jolkkonen J, Weise G, Posel C, Nitzsche B, et al. Concise review: Increasing the validity of cerebrovascular disease models and experimental methods for translational stem cell research. *Stem Cells*. 2017;35:1141-1153
7. Caroff J, King RM, Mitchell JE, Marosfoi M, Licwinko JR, Gray-Edwards HL, et al. Focal cooling of brain parenchyma in a transient large vessel occlusion model: Proof-of-concept. *J Neurointerv Surg*. 2020;12:209-213
8. Mordasini P, Brekenfeld C, Byrne JV, Fischer U, Arnold M, Jung S, et al. Experimental evaluation of immediate recanalization effect and recanalization efficacy of a new thrombus retriever for acute stroke treatment in vivo. *AJNR Am J Neuroradiol*. 2013;34:153-158

9. Cattaneo GF, Herrmann AM, Eiden SA, Wieser M, Kellner E, Doostkam S, et al. Selective intra-carotid blood cooling in acute ischemic stroke: A safety and feasibility study in an ovine stroke model. *J Cereb Blood Flow Metab*. 2021:271678X211024952
10. Werner P, Saur D, Zeisig V, Ettrich B, Patt M, Sattler B, et al. Simultaneous pet/mri in stroke: A case series. *J Cereb Blood Flow Metab*. 2015;35:1421-1425
11. Walczak P, Wojtkiewicz J, Nowakowski A, Habich A, Holak P, Xu J, et al. Real-time mri for precise and predictable intra-arterial stem cell delivery to the central nervous system. *J Cereb Blood Flow Metab*. 2017;37:2346-2358
12. Smith AJ, Clutton RE, Lilley E, Hansen KEA, Brattelid T. Prepare: Guidelines for planning animal research and testing. *Lab Anim*. 2018;52:135-141
13. Takagi S, Cocito L, Hossmann KA. Blood recirculation and pharmacological responsiveness of the cerebral vasculature following prolonged ischemia of cat brain. *Stroke*. 1977;8:707-712
14. Ebhardt G, Mies G, Auer LM, Traupe H, Heiss WD. Neuronal injury following permanent middle cerebral artery occlusion in cats. *Acta Neuropathol*. 1983;60:70-74
15. Heiss WD, Graf R, Wienhard K, Lottgen J, Saito R, Fujita T, et al. Dynamic penumbra demonstrated by sequential multitracer pet after middle cerebral artery occlusion in cats. *J Cereb Blood Flow Metab*. 1994;14:892-902
16. Jansen IGH, Mulder M, Goldhoorn RB, investigators MCR. Endovascular treatment for acute ischaemic stroke in routine clinical practice: Prospective, observational cohort study (mr clean registry). *BMJ*. 2018;360:k949
17. Ringelstein EB, Biniek R, Weiller C, Ammeling B, Nolte PN, Thron A. Type and extent of hemispheric brain infarctions and clinical outcome in early and delayed middle cerebral artery recanalization. *Neurology*. 1992;42:289-298

18. Purdy PD, Devous MD, White CL, Batjer HH, Samson DS, Brewer K, et al. Reversible middle cerebral-artery embolization in dogs without intracranial surgery. *Stroke*. 1989;20:1368-1376
19. Platt SR, Holmes SP, Howerth EW, Duberstein KJJ, Dove CR, Kinder HA, et al. Development and characterization of a yucatan miniature biomedical pig permanent middle cerebral artery occlusion stroke model. *Exp Transl Stroke Med*. 2014;6:5
20. Wells AJ, Vink R, Blumbergs PC, Brophy BP, Helps SC, Knox SJ, et al. A surgical model of permanent and transient middle cerebral artery stroke in the sheep. *PLoS One*. 2012;7:e42157
21. Marshall JWB, Cummings RM, Bowes LJ, Ridley RM, Green AR. Functional and histological evidence for the protective effect of nxy-059 in a primate model of stroke when given 4 hours after occlusion. *Stroke*. 2003;34:2228-2233
22. Herrmann AM, Cattaneo GFM, Eiden SA, Wieser M, Kellner E, Maurer C, et al. Development of a routinely applicable imaging protocol for fast and precise middle cerebral artery occlusion assessment and perfusion deficit measure in an ovine stroke model: A case study. *Front Neurol*. 2019;10:1113
23. Verbree J, Bronzwaer AS, Ghariq E, Versluis MJ, Daemen MJ, van Buchem MA, et al. Assessment of middle cerebral artery diameter during hypocapnia and hypercapnia in humans using ultra-high-field mri. *J Appl Physiol (1985)*. 2014;117:1084-1089
24. Kapoor K, Kak VK, Singh B. Morphology and comparative anatomy of circulus arteriosus cerebri in mammals. *Anat Histol Embryol*. 2003;32:347-355
25. Ashwini CA SR, Jayanthi KS. Comparative anatomy of the circle of willis in man, cow, sheep, goat, and pig. 2008:54-65

26. Qian Z, Climent S, Maynar M, Uson-Garallo J, Lima-Rodrigues MA, Calles C, et al. A simplified arteriovenous malformation model in sheep: Feasibility study. *AJNR Am J Neuroradiol*. 1999;20:765-770
27. Burbridge B, Matte G, Remedios A. Complex intracranial arterial anatomy in swine is unsuitable for cerebral infarction projects. *Can Assoc Radiol J*. 2004;55:326-329
28. Naumann DN, Beaven A, Dretzke J, Hutchings S, Midwinter MJ. Searching for the optimal fluid to restore microcirculatory flow dynamics after haemorrhagic shock: A systematic review of preclinical studies. *Shock*. 2016;46:609-622
29. Novak V, Chowdhary A, Farrar B, Nagaraja H, Braun J, Kanard R, et al. Altered cerebral vasoregulation in hypertension and stroke. *Neurology*. 2003;60:1657-1663
30. Strong AJ, Goodhardt MJ, Branston NM, Symon L. A comparison of the effects of ischaemia on tissue flow, electrical activity and extracellular potassium ion concentration in cerebral cortex of baboons. *Biochem Soc Trans*. 1977;5:158-160
31. Symon L. Regional vascular reactivity in the middle cerebral arterial distribution. An experimental study in baboons. *J Neurosurg*. 1970;33:532-541
32. Laughlin MH, Davis MJ, Secher NH, van Lieshout JJ, Arce-Esquivel AA, Simmons GH, et al. Peripheral circulation. *Compr Physiol*. 2012;2:321-447
33. Duncker DJ, Bache RJ. Regulation of coronary vasomotor tone under normal conditions and during acute myocardial hypoperfusion. *Pharmacol Ther*. 2000;86:87-110
34. Schmoker JD, Shackford SR, Zhuang J. The effect of lesion volume on cerebral vasomotor tone after focal brain injury and shock. *J Neurotrauma*. 1996;13:67-78
35. Zhang K, Sejnowski TJ. A universal scaling law between gray matter and white matter of cerebral cortex. *P Natl Acad Sci USA*. 2000;97:5621-5626

36. Falcao AL, Reutens DC, Markus R, Koga M, Read SJ, Tochon-Danguy H, et al. The resistance to ischemia of white and gray matter after stroke. *Ann Neurol*. 2004;56:695-701
37. Wang Y, Liu G, Hong D, Chen F, Ji X, Cao G. White matter injury in ischemic stroke. *Prog Neurobiol*. 2016;141:45-60
38. Hofman MA. Size and shape of the cerebral cortex in mammals. Ii. The cortical volume. *Brain Behav Evol*. 1988;32:17-26
39. Balsters JH, Zerbi V, Sallet J, Wenderoth N, Mars RB. Primate homologs of mouse cortico-striatal circuits. *Elife*. 2020;9
40. Stoll G, Nieswandt B. Thrombo-inflammation in acute ischaemic stroke - implications for treatment. *Nat Rev Neurol*. 2019;15:473-481
41. Moller K, Boltze J, Posel C, Seeger J, Stahl T, Wagner DC. Sterile inflammation after permanent distal mca occlusion in hypertensive rats. *J Cereb Blood Flow Metab*. 2014;34:307-315
42. Eckly A, Hechler B, Freund M, Zerr M, Cazenave JP, Lanza F, et al. Mechanisms underlying fecl3-induced arterial thrombosis. *J Thromb Haemost*. 2011;9:779-789
43. Kuluz JW, Prado R, He D, Zhao W, Dietrich WD, Watson B. New pediatric model of ischemic stroke in infant piglets by photothrombosis: Acute changes in cerebral blood flow, microvasculature, and early histopathology. *Stroke*. 2007;38:1932-1937
44. Murakami Y, Takamatsu H, Noda A, Osoda K, Nishimura S. Pet measurement of fk506 concentration in a monkey model of stroke. *Nucl Med Biol*. 2007;34:703-707
45. Ikeda S, Harada K, Ohwatashi A, Kamikawa Y, Yoshida A, Kawahira K. A new non-human primate model of photochemically induced cerebral infarction. *PLoS One*. 2013;8:e60037

46. d'Esterre CD, Aviv RI, Morrison L, Fainardi E, Lee TY. Acute multi-modal neuroimaging in a porcine model of endothelin-1-induced cerebral ischemia: Defining the acute infarct core. *Transl Stroke Res*. 2015;6:234-241
47. del Zoppo GJ, Copeland BR, Anderchek K, Hacke W, Koziol JA. Hemorrhagic transformation following tissue plasminogen activator in experimental cerebral infarction. *Stroke*. 1990;21:596-601
48. Del Zoppo GJ, Copeland BR, Harker LA, Waltz TA, Zyroff J, Hanson SR, et al. Experimental acute thrombotic stroke in baboons. *Stroke*. 1986;17:1254-1265
49. Bihel E, Pro-Sistiaga P, Letourneur A, Toutain J, Saulnier R, Insausti R, et al. Permanent or transient chronic ischemic stroke in the non-human primate: Behavioral, neuroimaging, histological, and immunohistochemical investigations. *J Cereb Blood Flow Metab*. 2010;30:273-285
50. Brooks OW, King RM, Nossek E, Marosfoi M, Caroff J, Chueh JY, et al. A canine model of mechanical thrombectomy in stroke. *J Neurointerv Surg*. 2019;11:1243-1248
51. Christoforidis GA, Rink C, Kontzialis MS, Mohammad Y, Koch RM, Abduljalil AM, et al. An endovascular canine middle cerebral artery occlusion model for the study of leptomeningeal collateral recruitment. *Invest Radiol*. 2011;46:34-40
52. Del Zoppo GJ, Copeland BR, Waltz TA, Zyroff J, Plow EF, Harker LA. The beneficial effect of intracarotid urokinase on acute stroke in a baboon model. *Stroke*. 1986;17:638-643
53. Golubczyk D, Kalkowski L, Kwiatkowska J, Zawadzki M, Holak P, Glodek J, et al. Endovascular model of ischemic stroke in swine guided by real-time mri. *Sci Rep*. 2020;10:17318
54. Maki T, Wakita H, Mase M, Itagaki I, Saito N, Ono F, et al. Watershed infarcts in a multiple microembolic model of monkey. *Neurosci Lett*. 2011;499:80-83

55. Lehmann KG, van Suylen RJ, Stibbe J, Slager CJ, Oomen JA, Maas A, et al. Composition of human thrombus assessed by quantitative colorimetric angioscopic analysis. *Circulation*. 1997;96:3030-3041
56. Robbie LA, Young SP, Bennett B, Booth NA. Thrombi formed in a chandler loop mimic human arterial thrombi in structure and rai-1 content and distribution. *Thromb Haemost*. 1997;77:510-515
57. Gralla J, Schroth G, Remonda L, Nedeltchev K, Slotboom J, Brekenfeld C. Mechanical thrombectomy for acute ischemic stroke: Thrombus-device interaction, efficiency, and complications in vivo. *Stroke*. 2006;37:3019-3024
58. Herrmann AM, Meckel S, Gounis MJ, Kringe L, Motschall E, Mulling C, et al. Large animals in neurointerventional research: A systematic review on models, techniques and their application in endovascular procedures for stroke, aneurysms and vascular malformations. *J Cereb Blood Flow Metab*. 2019;39:375-394
59. Fukuda S, del Zoppo GJ. Models of focal cerebral ischemia in the nonhuman primate. *Ilar J*. 2003;44:96-104
60. Boltze J, Forschler A, Nitzsche B, Waldmin D, Hoffmann A, Boltze CM, et al. Permanent middle cerebral artery occlusion in sheep: A novel large animal model of focal cerebral ischemia. *J Cereb Blood Flow Metab*. 2008;28:1951-1964
61. Imai H, Konno K, Nakamura M, Shimizu T, Kubota C, Seki K, et al. A new model of focal cerebral ischemia in the miniature pig. *J Neurosurg*. 2006;104:123-132
62. Mehra M, Henninger N, Hirsch JA, Chueh J, Wakhloo AK, Gounis MJ. Preclinical acute ischemic stroke modeling. *J Neurointerv Surg*. 2012;4:307-313
63. Marshall JWB, Ridley RM. Assessment of cognitive and motor deficits in a marmoset model of stroke. *Ilar J*. 2003;44:153-160

64. Moore TL, Killiany RJ, Pessina MA, Moss MB, Finklestein SP, Rosene DL. Recovery from ischemia in the middle-aged brain: A nonhuman primate model. *Neurobiol Aging*. 2012;33
65. Kang BT, Lee JH, Jung DI, Park C, Gu SH, Jeon HW, et al. Canine model of ischemic stroke with permanent middle cerebral artery occlusion: Clinical and histopathological findings. *J Vet Sci*. 2007;8:369-376
66. Tanaka Y, Imai H, Konno K, Miyagishima T, Kubota C, Puentes S, et al. Experimental model of lacunar infarction in the gyrencephalic brain of the miniature pig: Neurological assessment and histological, immunohistochemical, and physiological evaluation of dynamic corticospinal tract deformation. *Stroke*. 2008;39:205-212
67. Duberstein KJ, Platt SR, Holmes SP, Dove CR, Howerth EW, Kent M, et al. Gait analysis in a pre- and post-ischemic stroke biomedical pig model. *Physiol Behav*. 2014;125:8-16
68. Mack WJ, King RG, Hoh DJ, Coon AL, Ducruet AF, Huang J, et al. An improved functional neurological examination for use in nonhuman primate studies of focal reperfused cerebral ischemia. *Neurol Res*. 2003;25:280-284
69. Szabo D, Mills DS, Range F, Viranyi Z, Miklosi A. Is a local sample internationally representative? Reproducibility of four cognitive tests in family dogs across testing sites and breeds. *Anim Cogn*. 2017;20:1019-1033
70. Kornum BR, Knudsen GM. Cognitive testing of pigs (sus scrofa) in translational biobehavioral research. *Neurosci Biobehav Rev*. 2011;35:437-451
71. Morton AJ, Avanzo L. Executive decision-making in the domestic sheep. *PLoS One*. 2011;6:e15752

72. McBride SD, Perentos N, Morton AJ. A mobile, high-throughput semi-automated system for testing cognition in large non-primate animal models of huntington disease. *J Neurosci Methods*. 2016;265:25-33
73. Diehl KH, Hull R, Morton D, Pfister R, Rabemampianina Y, Smith D, et al. A good practice guide to the administration of substances and removal of blood, including routes and volumes. *J Appl Toxicol*. 2001;21:15-23
74. Goodman SL. Sheep, pig, and human platelet-material interactions with model cardiovascular biomaterials. *J Biomed Mater Res*. 1999;45:240-250
75. Siller-Matula JM, Plasenzotti R, Spiel A, Quehenberger P, Jilma B. Interspecies differences in coagulation profile. *Thromb Haemost*. 2008;100:397-404
76. Lemini C, Jaimez R, Franco Y. Gender and inter-species influence on coagulation tests of rats and mice. *Thromb Res*. 2007;120:415-419
77. Jin R, Yang G, Li G. Inflammatory mechanisms in ischemic stroke: Role of inflammatory cells. *J Leukoc Biol*. 2010;87:779-789
78. Overgaard NH, Fan TM, Schachtschneider KM, Principe DR, Schook LB, Jungersen G. Of mice, dogs, pigs, and men: Choosing the appropriate model for immuno-oncology research. *Ilar J*. 2018;59:247-262
79. Entrican G, Wattegedera SR, Griffiths DJ. Exploiting ovine immunology to improve the relevance of biomedical models. *Mol Immunol*. 2015;66:68-77
80. Laber K WM, Bingel S, Goodrich J, Smith A, Swindle M. Biology and diseases of swine. *Laboratory Animal Medicine*. 2002:615-673
81. Bjornson-Hooper ZB FG, Spitzer MH, Madhireddy D, McIlwain D, Nolan GP. A comprehensive atlas of immunological differences between humans, mice and non-human primates. *bioRxiv*. 2019

82. Gimsa U, Tuchscherer M, Kanitz E. Psychosocial stress and immunity-what can we learn from pig studies? *Front Behav Neurosci*. 2018;12:64
83. Luo L, Jansen CA, Bolhuis JE, Arts JAJ, Kemp B, Parmentier HK. Early and later life environmental enrichment affect specific antibody responses and blood leukocyte subpopulations in pigs. *Physiol Behav*. 2020;217:112799
84. Clarke MR, Harrison RM, Didier ES. Behavioral, immunological, and hormonal responses associated with social change in rhesus monkeys (macaca mulatta). *Am J Primatol*. 1996;39:223-233
85. Byrom MJ, Bannon PG, White GH, Ng MK. Animal models for the assessment of novel vascular conduits. *J Vasc Surg*. 2010;52:176-195
86. Pichler L. Parameters of coagulation and fibrinolysis in different animal species - a literature based comparison. *Wien Tierarztl Monat*. 2008;95:282-295
87. Nudo RJ, Milliken GW. Reorganization of movement representations in primary motor cortex following focal ischemic infarcts in adult squirrel monkeys. *J Neurophysiol*. 1996;75:2144-2149
88. Virley D, Hadingham SJ, Roberts JC, Farnfield B, Elliott H, Whelan G, et al. A new primate model of focal stroke: Endothelin-1-induced middle cerebral artery occlusion and reperfusion in the common marmoset. *J Cereb Blood Flow Metab*. 2004;24:24-41
89. Vasquez CA, Moen SL, Juliano MJ, Jagadeesan BD, Pluhar GE, Chen CC, et al. Development of a novel canine model of ischemic stroke: Skull base approach with transient middle cerebral artery occlusion. *World Neurosurg*. 2019;127:e251-e260
90. Sakoh M, Rohl L, Gyldensted C, Gjedde A, Ostergaard L. Cerebral blood flow and blood volume measured by magnetic resonance imaging bolus tracking after acute stroke in pigs: Comparison with [(15)o]h(2)o positron emission tomography. *Stroke*. 2000;31:1958-1964

91. Arikan F, Martinez-Valverde T, Sanchez-Guerrero A, Campos M, Esteves M, Gandara D, et al. Malignant infarction of the middle cerebral artery in a porcine model. A pilot study. *PLoS One*. 2017;12:e0172637
92. Hudgins WR, Garcia JH. Transorbital approach to the middle cerebral artery of the squirrel monkey: A technique for experimental cerebral infarction applicable to ultrastructural studies. *Stroke*. 1970;1:107-111
93. West GA, Golshani KJ, Doyle KP, Lessov NS, Hobbs TR, Kohama SG, et al. A new model of cortical stroke in the rhesus macaque. *J Cereb Blood Flow Metab*. 2009;29:1175-1186
94. Cook DJ, Teves L, Tymianski M. Treatment of stroke with a psd-95 inhibitor in the gyrencephalic primate brain. *Nature*. 2012;483:213-217
95. Marshall JW, Ridley RM. Assessment of functional impairment following permanent middle cerebral artery occlusion in a non-human primate species. *Neurodegeneration*. 1996;5:275-286
96. Hirouchi Y, Suzuki E, Mitsuoka C, Jin HR, Kitajima S, Kohjimoto Y, et al. Neuroimaging and histopathological evaluation of delayed neurological damage produced by artificial occlusion of the middle cerebral artery in cynomolgus monkeys - establishment of a monkey model for, delayed cerebral ischemia -. *Exp Toxicol Pathol*. 2007;59:9-16
97. Deley G, Weyne J, Demeester G, Stryckmans K, Goethals P, Vandevelde E, et al. Experimental thromboembolic stroke studied by positron emission tomography - immediate versus delayed reperfusion by fibrinolysis. *J Cerebr Blood F Met*. 1988;8:539-545

98. Shaibani A, Khawar S, Shin W, Cashen TA, Schirf B, Rohany M, et al. First results in an mr imaging-compatible canine model of acute stroke. *Am J Neuroradiol*. 2006;27:1788-1793
99. Kito G, Nishimura A, Susumu T, Nagata R, Kuge Y, Yokota C, et al. Experimental thromboembolic stroke in cynomolgus monkey. *J Neurosci Meth*. 2001;105:45-53
100. Levy EI, Hanel RA, Tio FO, Garlick DS, Bailey L, Cunningham MR, et al. Safety and pharmacokinetics of sirolimus-eluting stents in the canine cerebral vasculature: 180 day assessment. *Neurosurgery*. 2006;59:925-933
101. Schwartz AE, Pile-Spellman J. New model of reperfused stroke by occlusion of the anterior cerebral artery in baboons. *Acta Neurochir*. 2011;153:327-331
102. Gao H, Liu Y, Lu S, Xiang B, Wang C. A reversible middle cerebral artery occlusion model using intraluminal balloon technique in monkeys. *J Stroke Cerebrovasc Dis*. 2006;15:202-208
103. Freret T, Bouet V, Toutain J, Saulnier R, Pro-Sistiaga P, Bihel E, et al. Intraluminal thread model of focal stroke in the non-human primate. *J Cereb Blood Flow Metab*. 2008;28:786-796
104. Cook DJ, Teves L, Tymianski M. A translational paradigm for the preclinical evaluation of the stroke neuroprotectant tat-nr2b9c in gyrencephalic nonhuman primates. *Sci Transl Med*. 2012;4:154ra133

Figure Legends

Figure 1. Antero-posterior (panel A) and lateral (panel B) view of 3-D angiography showing *rete mirabile epidurale rostrale* (RM), middle cerebral arteries (MCA) and ascending pharyngeal arteries (APA) in swine.

Figure 2. Comparative overview of circle of Willis (CoW) in humans, dogs, swine, sheep and NHP. In men, the CoW is composed of the left and right anterior cerebral artery (ACA), the anterior communicating artery (ACoA), left and right internal carotid arteries (ICA), left and right posterior cerebral arteries (PCA), as well as left and right posterior communicating arteries (PCoA). The CoW in dogs and NHP is relatively similar to humans with the exception of an absent ACoA. Instead, both ACAs merge into a single median ACA, which then divides into right and left branches. This anatomical variation also exists in human anatomy and is known as 'azygos ACA'. As a variation, swine and sheep can have a fine plexiform network of vessels between the two ACAs, instead of one complete ACoA. The PCA branches off the PCoA in swine and sheep whereas it branches off the basilar artery in humans. The anterior cerebellar artery in dogs and sheep is a branch of the PCA, whereas a similar artery arises from the basilar artery in primates and humans, namely the anterior inferior cerebellar artery. Anatomical variations in the CoW exist in both humans and animals and an incomplete circle is associated with a reduction of the compensatory ability and a higher risk of stroke.

Figure 3. Flowchart describing the most relevant aspects to take into account when choosing a large animal model of AIS. For perfusion studies requiring direct visualization of the tissue, we advise craniectomy and consequently an external/surgical approach rather than an endovascular approach. However, in general, the choice between external/surgical or endovascular should be based on the specific expertise of the research center. Moreover, when infrastructure, funding and ethical approval are available for NHP models, we

recommend their use due to the anatomical and physiological similarities to humans. However, for proof-of-concept, descriptive, or non-stroke EVT studies, we recommend using sheep and swine models due to wider availability, less demanding housing requirements, and lower associated costs compared to dog and NHP models. When studying EVT in a thromboembolic stroke model, dogs and NHP are the only options due to the rete mirabile in swine and sheep. CV indicates cerebrovascular; EVT, endovascular treatment; and NHP, non-human primate.

Tables

Table 1. Feasiblity and implementation of occlusion techniques in large animal models of acute ischemic stroke.

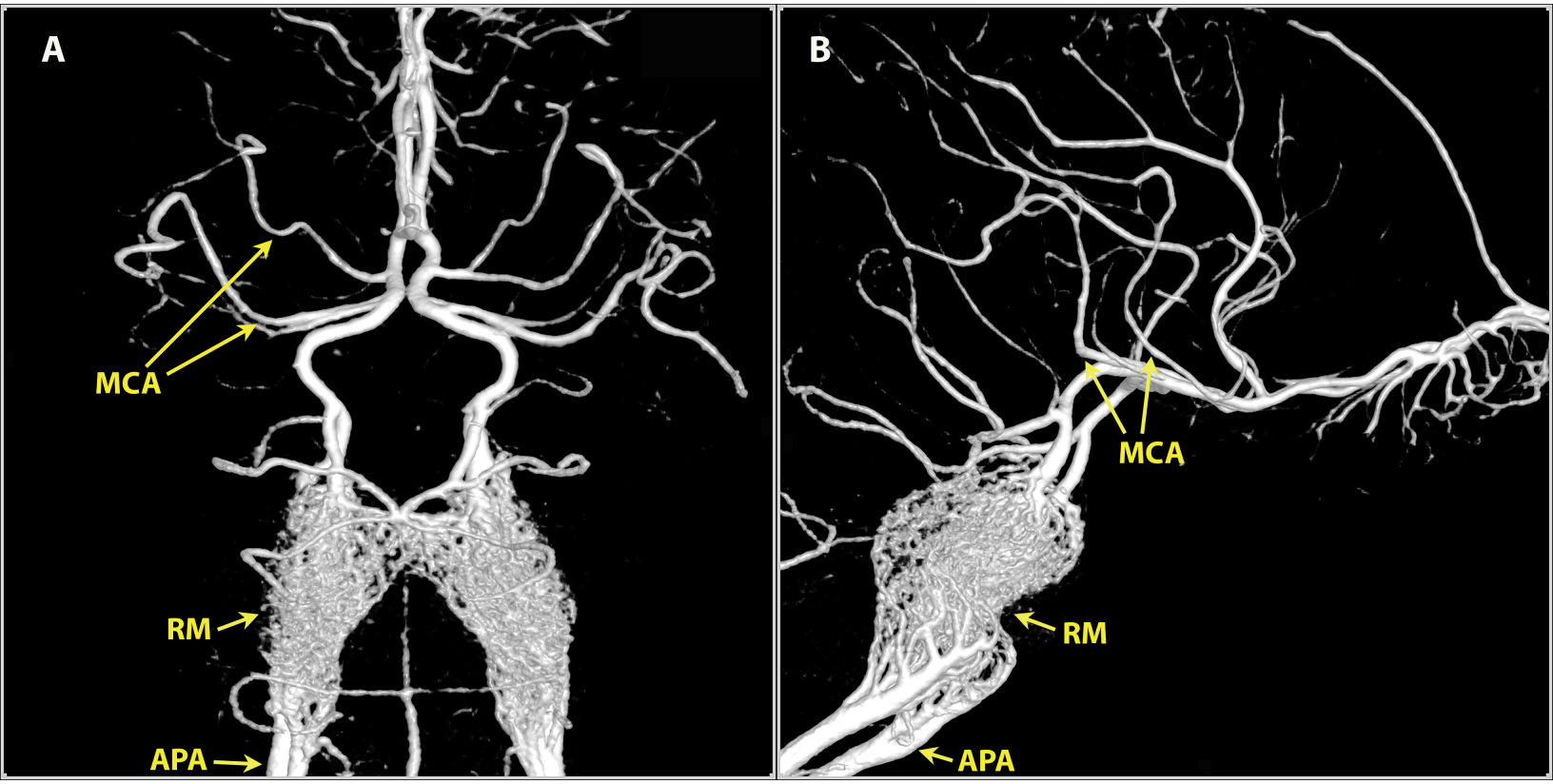
Occlusion technique	Dog	Swine	Sheep	NHP
Surgical				
Clipping	✓[89]	✓[66, 90, 91]	✓[20]	✓[92-94]
Electrocoagulation	✓[89]	✓[61, 90]	✓[20, 60]	✓[21, 95]
Ligation	✓	✓	✓	✓[96]
Chemical thrombosis	✓	✓[43]	✓	✓[44, 45]
Endothelin-1 injection	✓	✓[46]	✓	✓[88]
Endovascular				
Thromboembolic	✓[50, 97, 98]	-	-	✓[99]
Silicone plugs	✓[18, 100]	-	-	✓
Endovascular coils	✓[51]	-	-	✓[64, 101]
Balloon occlusion	✓	-	-	✓[52, 102]
Nylon threads	✓	-	-	✓[49, 103]
Microbeads	✓	-	-	✓[54, 104]
Thrombin injection	✓	✓[53]	✓	✓

The feasibility of performing each occlusion technique in each of the different animal models is indicated with ✓. References are added as an example in those models where the technique has been implemented and reported, but we do not give a complete overview of the available literature. A hyphen (-) indicates lack of feasibility of the occlusion technique due to the presence of rete mirabile. NHP indicates non-human primate.

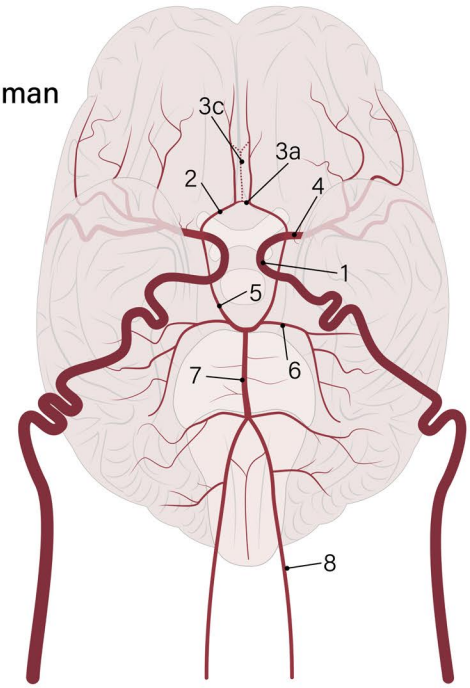
Table 2. Strengths, limitations and challenges of large animal stroke models.

Animal model	Dog	Swine	Sheep	NHP
Anatomy and physiology				
Cerebrovascular anatomy				
External approach	+ +	+	+	+ +
Endovascular approach	+/-	- -	- -	+ +
Brain structure	+	+	+	+ +
Coagulation	+	+	+ +	+
Modeling techniques				
External approach*	+	+	+	+
Endovascular approach	+	- -	- -	+ +
Reproducible infarct size				
External approach	+	+	+	+
Endovascular approach	-	- -	- -	-
Procedural complications				
External approach	+	+	+	+
Endovascular approach	-	- -	- -	+
Functional testing	+	+	+	+
Implementation aspects				
Housing	-	+	+	-
Costs	+	+ +	+ +	-
Expertise and infrastructure†				

* The external approach can be achieved transorbitally or transcranially. For NHP, the preferred external approach is transorbital, as it involves less postoperative care. † Expertise and infrastructure are not scored as they differ per research facility. They can be scored by each center individually as it should be taken into account when deciding which animal model to use. + + = very beneficial. + = beneficial. +/- = slightly challenging. - = challenging. - - = very challenging / not possible. NHP indicates non-human primates.

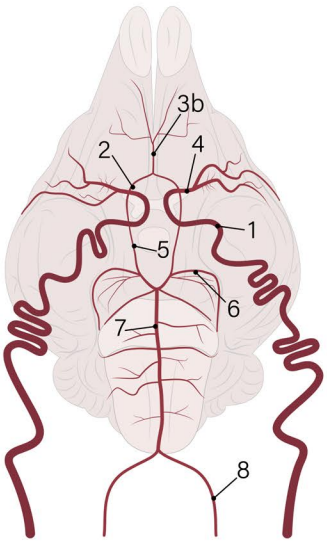

A
MCA
RM
APA
B
MCA
RM
APA

Human

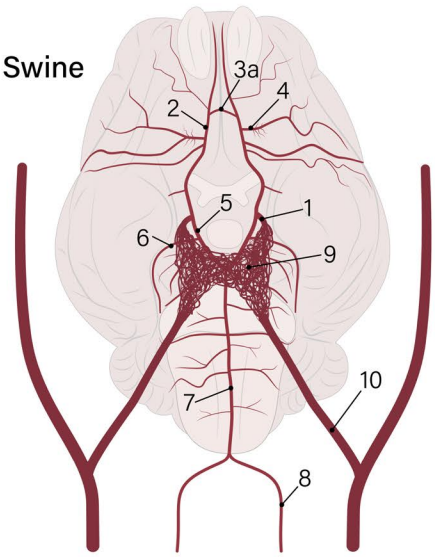


1. **ICA** Internal Carotid Artery
2. **ACA** Anterior Cerebral Artery
3a. **ACoA** Anterior Communicating Artery
3b. **mACA** Median Anterior Cerebral Artery
3c. **AzCA** Azygos Anterior Cerebral Artery
4. **MCA** Middle Cerebral Artery
5. **PCoA** Posterior Communicating Artery
6. **PCA** Posterior Cerebral Artery
7. **BA** Basilar Artery
8. **VA** Vertebral Artery
9. **RM** *Rete Mirabile Epidurale Rostrale*
10. **APA** Ascending Pharyngeal Artery

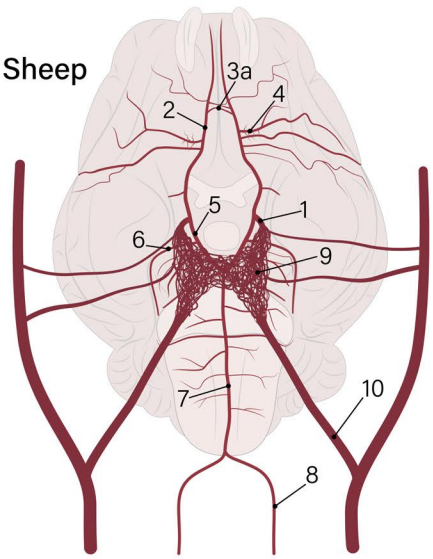
Dog



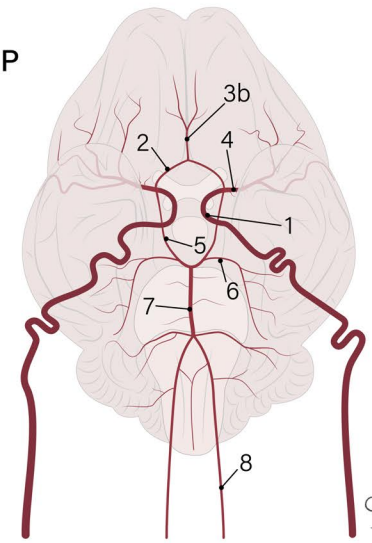
Swine



Sheep



NHP



qbig 2021

Large-animal Models for Acute Ischemic Stroke

Area of Study

CV Pathophysiology

Therapeutic Interventions

Perfusion

Thrombosis

Inflammation

Neuroprotection

EVT (+/- thrombolysis)

Direct Tissue Visualization

Yes

No

Thromboembolic Stroke for EVT

No

Yes

Specific Expertise of the Center

Methodology

External/Surgery

Endovascular

Preferably for Exploratory Studies

Preferably

Infrastructure, Funding and Ethical Approval for Dog/NHP?

No

Yes

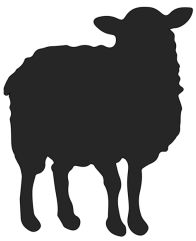
Sheep/Swine

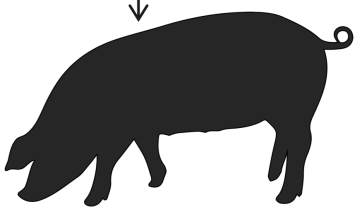
Infrastructure and Expertise

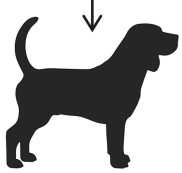
Infrastructure, Funding and Ethical Approval for NHP?

No

Yes







Sheep

Swine

Dog

NHP