Standard operating procedures (SOP) in experimental stroke research: SOP for middle cerebral artery occlusion in the mouse

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Thus far, the translation of promising results from preclinical stroke research into effective clinical therapy has not met with success *(1)*. Among the numerous possible reasons for this failure, quality problems in some of the basic research or preclinical studies have to be considered. False-positive results, inflated effect sizes, and marginal reproducibility may have overestimated or even affected the potential of novel stroke therapeutics *(2)*. Systematic reviews have found quantitative evidence that low study quality may have introduced a bias into preclinical stroke research *(3,4,5)*. As opposed to many other causes of the 'translational roadblock', study quality is fully under the control of the researcher, and thus a prime target for improvement. Increasingly, funding bodies and review boards overseeing animal experiments are taking a proactive stance, and demand auditable measures of quality control in preclinical research *(6)*. The Stroke Therapy Academic Industry Roundtable (STAIR) recently updated its recommendations for the evaluation of preclinical data on neuroprotective drugs *(7)* to include good laboratory practice (GLP) issues *(8)*.
Monitoring, auditing, and standard operating procedures (SOPs) are key elements of quality control in randomized clinical trials (RCTs). It has been proposed that experimental stroke research adapt some of the tools used in clinical stroke research. In particular, stroke laboratories should set up and publish their SOPs (e.g., on their institutional websites), and guarantee that their studies adhere to these standards *(9)*. This is all the more important, as a certain portion of their experiments, evaluations, etc. are not performed by professionals, but rather by students in training who are unaware of these issues.

A standard operating procedure (SOP) is a set of instructions with the force of a directive covering those features of operations that lend themselves to a definite or standardized procedure without loss of effectiveness. The primary purpose of an SOP in experimental stroke research is to guide and standardize working procedures in order to ensure data reliability and integrity. It is crucial that researchers, students and technicians read and follow the SOPs. If this is not the case, SOPs will not only fail to achieve their goal; they will also engender a false sense of security. Failures are often due to technical shortcomings in the SOPs themselves. SOPs should be written by the user, as they must convey a clear instruction. The user must not only understand the instruction but also be prepared to carry it out.

We in the following introduce for the first time an SOP in experimental stroke research.
Write down what you do, do what is written down!

SOP - Middle cerebral artery occlusion (MCAO) in the mouse (intraluminal suture)

1 Aim

Experimental induction of focal cerebral ischemia after occlusion of the middle cerebral artery in mice. Applies to a procedure in a standard lab equipped and certified for in vivo experimentation in mice (including anaesthesia with volatile anaesthetics). Experimental procedures require approval by the relevant committees.

2 Background

Experimental focal ischemia is most commonly studied after permanent or transient occlusion of the middle cerebral artery (MCA) in rodents. Proximal MCA occlusion can be induced by an intraluminal suture (so-called filament model) and causes injury to cortex and deeper brain structures (striatum). Distal MCA occlusion (the so-called 'Brint' or 'Tamura'-models) is usually produced by blocking the MCA after it gives off lencticulostriate branches at the basal surface of the lateral part of the cerebral hemisphere. Distal occlusion typically spares the striatum and primarily involves the neocortex. If recirculation is established early (2 hrs or less) outcome is better (transient MCA occlusion). In some ways, the reperfused brain imitates restoration of blood flow after spontaneous lysis of a thromboembolic clot in humans, even though reperfusion after clot lysis is certainly more complex than an on-off phenomenon as modelled by placement and retraction of an intravascular filament.

3 Scope

Department of Experimental Neurology (all employees)

4 Process description

4.1 Materials & Instrumentation

- Ready coated MCAo filaments, e.g. Doccol® or 8/0 nylon monofilament for coating with silicone gel with hardener (e.g. Xantopren®)
- surgical thread 4/0- 7/0
- surgical needle and thread for skin suture
- dissecting microscope (max. x 40)
- temperature feedback controlled heating plate
- surgical instruments
- forceps (e.g. Dumont Medical #5 straight tip 0,05mm x 0,02 mm)
- surgical scissors (skin cut)
- forceps for skin handling and suture (e.g. standard anatomical)
- vascular spring scissors (e.g. Vannas)
- absorbing material (e.g. swab)
- Micro vascular clamp (e.g. S&G B1-V) and applying forceps
- Needle holder (e.g. Olsen-Hegar or other)
- anaesthesia system for isoflurane, oxygen and nitrous oxide
- heated recovery cage

4.2 Cautions

Maintain a body temperature of 37.0 +/- 0.5 °C during occlusion and after reperfusion (for 2 hours).
Ensure proper pain relief in the perioperative and postoperative period, e.g. by repeated topical application of a long-acting local anaesthetic, for instance bupivacaine ointment serving as an absorption depot.
For specific details see Maximising animal welfare in experimental rodent stroke SOP.
Surgical procedures should be carried out under clean conditions. (disinfected surgical instruments and materials, clean gown, gloves, etc.). See Aseptic technique and anaesthesia SOP and Appendix below.

4.3 Personnel and Qualifications

In general, surgeons need:

- general supervision and instruction
- the appropriate certification
- official registration/approval by appropriate authorities

4.4 Protocol

General

1. Mice are anaesthetized with 1.5-3.5% Isoflurane and maintained in 1.0-2.5% Isoflurane with approximately 75/25 N2O/O2. For additional analgesic treatment follow the Maximizing animal welfare in experimental rodent stroke SOP.2. The use of any non-invasive physiological monitoring available locally (e.g. Laser Doppler) is recommended

3. Maintain body temperature at 37.0 +/- 0.5 °C using a heating plate or homoeothermic blanket system.

4. Place the mouse in supine position. Disinfect the skin of the ventral neck.

MCAO Surgery:

5. In our lab the left MCA will be occluded routinely. Right MCA occlusion is also permitted. Left or right must be defined in advance and reported with your data. Reasons to change sides could be planned behavioural experiments to determine functional outcome more robustly, like paw preference or the handedness of the surgeon. The following procedure describes left MCA occlusion.

6. A midline neck incision is made and the soft tissues are pulled apart.

7. The common carotid artery (CCA) is carefully dissected free from the surrounding nerves (without harming the vagus nerve) and a ligature is made using suture thread.
8. Then the external carotid artery (ECA) on the same side is separated and a second knot is made.
9. Next, the internal carotid artery (ICA) is isolated and a loose knot is prepared with a suture thread.
10. After obtaining good view of the internal carotid artery (ICA) and the pterygopalatine artery (PA), both arteries are clipped.
11. A small hole is cut in the CCA before it bifurcates to the ECA and the ICA.
12. A Doccol® filament or a monofilament made of 8/0 nylon coated with silicon hardener mixture is then introduced into the artery. Doccol® filaments should be disinfected with 70% ethanol or sterilized with ethylene oxide. Alternative filaments should be sterilized according to local protocol.
13. The clipped arteries are opened while the filament is inserted into the ICA to occlude MCA.
14. The third knot on the ICA is closed to fix the filament in position.
15. Ischemia is confirmed by Laser Doppler flowmetry, MRI, or by withdrawing general anaesthesia and checking for neurological deficit according to local protocols, like circling behaviour during occlusion time, and twisting of the mice when held by the tail.
16. The mice are allowed to recover in the heated recovery cage at thermoneutral temperature (30-31°C) for the duration of the MCA occlusion.
17. After X min/hours occlusion, the mice are re-anaesthetized and the third knot is opened and the filament withdrawn (if reperfusion is intended).
18. The remaining threads are cut and the skin is adapted with a surgical suture
19. Rehydration and pain relief. Refer to the Maximizing animal welfare in experimental rodent stroke SOP and follow one of the recommended protocols.

Sham procedure:

For sham operations the filament is inserted to occlude the MCA and withdrawn immediately to allow instant reperfusion (12.). The subsequent operation is identical to the animals undergoing cerebral ischemia (13.- 19.).

A video is available which demonstrates the above described procedure. (see reference 10)

5 Evaluation / Control

no record

6 Modification

Description of modification in relation to last version (short).

part / chapter	description of modification
2, 4, 7	Clarification of procedures

7 Appendix to SOP

1. Entry qualification experiment for mouse MCAO surgeons:
New surgeons need to demonstrate in a series of experiments that they perform the MCAO-operation within 15 minutes. Reproducibility is verified by induction of a certain infarct volume within a standard deviation of 40%. Mortality must not exceed 10% within 24 hours.

2. Randomized selection of animals from cage and concealment of treatment allocation

2 a. Pharmacological study: Animals in cage are marked with an identity code at the beginning of the procedure. Computer program (random number generator) selects animal, and assigns it to concealed treatment arm ('A', 'B', etc.).
Stock solution or pharmaceutical ready for application is prepared by assistant and randomly assigned code ('A', 'B', etc.).

2 b. Genetically manipulated animals: Animals in both cages (e.g. knockout / wildtype) are marked with bar/dot code at the beginning of the procedure. Computer program (random number generator) selects animal and assigns it to concealed experimental arm ('A', 'B', etc.). Blinded intervention whenever possible.

3. Temperature control:
The body temperature of mice during surgery is maintained at 37.0 +/- 0.5 °C using a temperature-controlled heating plate. Maintain a body temperature of 37.0 +/- 0.5 °C also after reperfusion (for 2 hours) using a heated recovery box set at thermoneutral temperature (30-31°C).

4. Outcome assessment
Infarct volume should be evaluated blinded. Functional outcome, including a battery of tests to detect sensorimotor deficits should be assessed as well. Mortality and exclusion of animals have to be reported, including specific causes for exclusion.

For exclusion follow the main criteria:
No stroke, indicated by absence of functional deficit like circling behaviour during occlusion time, Insufficient Doppler flow reduction or missing functional deficit or missing infarct in MRI (DWI or T2) at 24h after MCAo.
Insufficient or non-existent reperfusion (insufficient restoration of Doppler flow).
Technical problems during induction of MCAo (excessive bleeding, prolonged operation time > 15 min, thread misplacement).
CAVE:
Especially in genetically manipulated animals, be aware of vascular alterations, which might directly affect stroke outcome

5. Physiological parameters:
Mean arterial blood pressure (MABP), heart rate (HR), blood gases, CBF should be measured in selected animals.

6. Quasi-sterile surgery
Prior to surgery, the surgeon has to clean his hands. The surgeon has to wear a lab coat, during surgery and it is advisable to wear hat, surgical mask and non-sterile gloves at all times the animal is being handled. If gloves cannot be used, a surgical hand disinfection should precede the operation. The necessary components of aseptic techniques in rodents include also disinfected instruments, and separate surgical and animal prep areas. The use of glass bead sterilizer for re-sterilization of instruments during for repetitive procedures is recommended.

- Instruments should be kept clean and sterile prior surgery (either by placing in 70% ethanol or a glass bead sterilizer for the appropriate period of time for the method used to be effective).
- Separate instruments should be used for skin and tissue handling.
- Instruments should be cleaned of blood and debris between surgeries. (Best: distilled water)
- If contamination has occurred, instruments must be cleaned between animals. Surgical gloves and blades should be changed after contamination.
- Following surgery all instruments must be thoroughly cleaned.

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