The effect of anesthetics on neurological outcome in preclinical rodent models of transient focal cerebral ischemia.

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Background
Recent retrospective studies of endovascular therapy for acute ischemic stroke have reported that general anesthesia and/or low blood pressures are risk factors for poor outcome. Decreases in blood pressure, with their potential to reduce perfusion to penumbral brain regions are certainly a plausible cause for concern, leading some clinicians to avoid general anesthesia unless it is indicated for patient safety. There remain some patients for whom general anesthesia is required. For the latter patients, it would be helpful to know whether some anesthetics possess intrinsic neuroprotective or neurotoxic properties, or whether it is sufficient to base anesthetic choice on the hemodynamic profiles of the individual drugs.

Aim
The purpose of this systematic review is to identify experiments reporting the effect of anesthetic administration (either administered pre- or post ischemia) during temporary focal ischemia on the histological outcome in preclinical models in rats or mice, and to evaluate the validity of the retrieved data. The primary outcomes that will be used are histological and neurological outcomes.

Search Strategy
Electronic search of “Medline” and “Embase”
MedLine

[<stroke$.tw.kw> OR <stroke$.ti.kw> OR <brain ischemia$.tw.kw> OR <transient ischemic attack$.tw.kw> OR <middle cerebral artery.tw.kw> OR <cerebral infarction$.tw.kw> OR <neuroprotective agent$.tw.kw>]

AND

[<propofol.mp.> OR <ketamine.mp> OR <sevoflurane.mp> OR <isoflurane.mp> OR <etomidate.mp> OR <halothane.mp.>]

Embase

[<stroke$.tw.kw> OR <stroke$.ti.kw> OR <brain ischemia$.tw.kw> OR <transient ischemic attack$.tw.kw> OR <middle cerebral artery.tw.kw> OR <cerebral infarction$.tw.kw> OR <neuroprotective agent$.tw.kw>]

AND

[<propofol.mp.> OR <ketamine.mp> OR <sevoflurane.mp> OR <isoflurane.mp> OR <etomidate.mp> OR <halothane.mp.>]

Inclusion Criteria

Studies identified by the search strategy will be evaluated independently by two investigators in two stages. Initial screening will be performed to capture relevant publications - for these a paper copy will be generated. Detailed review of the full papers will then identify the reports that will be coded in detail. Disagreements between investigators will be resolved by consensus after discussion. Included studies will report the effect of anesthetic administration on brain subjected to temporary focal ischemia induced by occlusion of the middle cerebral artery on histological and/or neurological outcomes. Included species will be rats and mice.

Exclusion Criteria

Studies will be limited to models of temporary focal cerebral (see appendix); permanent focal ischemia, forebrain ischemia, global cerebral ischemia and cellular/tissue models of ischemia will be excluded.
**Second exclusion**
Studies will be excluded if the effect size of the anesthetic intervention cannot be expressed as a mean and standard deviation, if there is no control intervention, and when the number of experimental subjects cannot be determined.

**Data Collection**
For included studies, the following information will be entered into an excel spreadsheet and RevMan 5.3 data manager:
Reference Identification: authors, year of publication, source (journal, abstract), funding source, conflict of interest statement, regulatory approval
Nature of Subjects: species/strain, age, weight, sex
Anesthetic Information: study drug (dose, timing of administration), dose-response design, control drug or awake design.
Induction of Ischemia: ischemia model, target of ischemia, timing of intervention, confirmation with CBF measurements, duration of ischemia, location of occlusion, infarct location.
Outcome Assessment: histological and/or neurological outcomes

**Quality Assessment**

Upon initial evaluation of study quality using the 10-point CAMARADES checklist, several criteria presented with high compliance (> 95%). These included, publication in a peer-reviewed journal, statement of temperature control and statement regarding regulatory compliance. Conversely, < 5% of studies presented with a formal sample size calculation. As such, these four variables would contribute little to the explanation of study heterogeneity and were omitted from
the CAMARADES checklist. Additionally, the metric associated with the avoidance of anesthetics with marked intrinsic properties was omitted given the subjective nature of this criterion. A modified CAMARADES checklist is thus presented and applied to all studies that includes the five remaining criteria of the original CAMARADES checklist plus an additional criterion as to whether the study investigated possible metabolic mechanisms. The additional of the metabolic mechanism criterion further serves to act as a surrogate for sample size calculations.

Study funding source will also be included. From this, plots will be developed to understand the relationship between standardized effect size and quality of study for each anesthetic drug evaluated. Two investigators will assess study quality independently.

**Outcome Data**

- Histological outcome: brain infarct size, apoptotic neurons by caspase-staining area of blood-brain-barrier breakdown by Evans blue staining.
- Neurological outcome: neurological outcome scores

Outcome data will be divided into acute and long-term categories based on time of assessment from ischemia initiation. Evidence for cellular mechanisms were extracted, but not subjected to meta-analysis.

**Methods of Data Extraction**

Reported mean values and standard deviations or standard errors will be used directly. When the latter are not available, these values will be obtained from graphs or figures after digitizing and calibrating 'snapshots' obtained from digital copies of the manuscripts. Graphical data will be extracted using digital ruler software. In the case of missing data or illegible graphs from which data extraction is not possible, authors will be contacted and the data was requested. If a response is not received, the study will be excluded from analysis. In the case of data reported as individual values, the data will evaluated for normality with the Shapiro-Wilk test. Data that fails the Shapiro-Wilk test, and data that was reported as median values with minimum, maximum, 25th, 75th centiles will be converted to mean and standard deviation by application of the methods of Wan, Hozo, and Bland\(^1,2\).
In some studies, the infarct volume will be reported separately for the cortex and the caudate putamen. Composite mean and standard deviation will be calculated as described the Cochrane handbook (handbook.cochrane.org/chapter...7_7_3_8).

**Statistical Analysis**

The primary outcome variables will be the pooled standardized mean difference in infarct volume and neurological outcome between treatment and control groups. This will be presented individually for each included anesthetic.

For reports that match one control group to multiple study groups, the number of subjects in the control group will be subjected to the Bonferroni correction, without changing the standard deviation in the controls\(^3\). The number of subjects in the study groups remains unaltered for the calculation of effect size. In dose-response studies, a dose of 1 MAC of anesthetic will be selected to represent the entire study; no Bonferroni correction will applied.

The outcome variables will be considered to be continuous and will be analyzed with a random effects model using the standardized mean difference with Hedge’s correction for small sample sizes. Moderator effects (drug, control anesthetic, and time of assessment) will be assessed by subgroup meta-analysis. The I\(^2\) metric will be used to assess heterogeneity. Publication bias will be investigated individually for each included anesthetic (e.g. each moderator) using funnel plots.
Appendix

Accepted duration of temporary focal ischemia: 50 minutes - 3 hours

Assessment Time Post-reperfusion

- Acute: 6 hours - ≤7 days
- Long-term: >7 - 28 days

Included Anesthetics

- Isoflurane
- Propofol
- Sevoflurane
- Ketamine
- Etomidate
- Halothane

Reference List

1. Hozo SP, Djulbegovic B, Hozo I: Estimating the mean and variance from the median, range, and the size of a sample. BMC Medical Research Methodology 2005; 5: