FULL TITLE: No Benefit of Hypertonic Saline Following Severe Experimental Intracerebral Hemorrhage

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Study Name: Hypertonic Saline following ICH

Experimental Plan (by Experiment):

- Experiment 1
    - Aim: Will 3% hypertonic saline (HTS) worsen bleeding at 8 hours post-stroke in a collagenase-induced rodent model of ICH?
        - Whether n= 12 rats per group receive 2mL/kg[4–6] IV boluses of 3% HTS or vehicle (0.9% saline solution).
    - Dependent variables
        - Hematoma volume (Primary endpoint), pain, cardiac samples will be taken for use later on
    - Hypothesis
        - We hypothesize that 3% HTS will not affect hematoma volume or other safety measures after ICH.
    - Timeline
        - On day 0, an ICH will be induced (3μL COL of 0.2U/μL in saline, or ~70μL bleed).[1]
        - HTS will be given via an IV bolus to the tail vein at 2 hours post-stroke.[2]
        - Pain will be assessed via rodent grimace scale immediately before and after HTS injection.
        - Rats will be euthanized at 8 hours post-stroke and assessed with hemoglobin assays for intraparenchymal hemorrhage volume.
    - Next steps
        - If HTS affected bleeding, further replications and experiments will be performed (e.g., looking at later interventions).
        - If bleeding was not affected, proceed to experiment 2.
- Experiment 2
    - Aim: Will 3% HTS lessen edema at 24 hours in a collagenase-induced rodent model of ICH?
    - There are two independent variables
        - Whether n=20 rats receive 2mL/kg IV boluses of 3% HTS or vehicle (0.9% saline solution)
    - Dependent variables
        - Edema (Primary endpoint), behaviour (NDS)
    - Hypothesis
        - We hypothesize that HTS will significantly reduce edema following hemorrhagic stroke, and that this will also result in improved behavioural outcome.

- Timeline
    - All rats will receive 2 ten-minute handling sessions and an NDS training session prior to baseline.
    - 2 days prior to ICH, an NDS baseline will be performed.
    - On day 0, an ICH will be induced (3μL COL of 0.2U/μL in saline, or ~70μL bleed).[1]
    - HTS will be given via an IV bolus to the tail vein every 12 hours, starting at 2 hours post-stroke (i.e., 2 and 14 hours post-stroke).[2]
    - NDS will be assessed at 24 hours post-stroke.
- Next steps
    - If a benefit on edema was found, this experiment will be repeated with a 72 hour endpoint with ICP monitoring.

- Experiment 3
    - Aim: Will HTS affect cellular shrinkage or sub-cellular health at 24 hours in a collagenase-induced rodent model of ICH?
    - Independent variable
        - Whether n=12 rats per group receive 3% HTS or vehicle (0.9% saline).
    - Dependent variables
        - Cell volume (Primary endpoint) and cell density for ipsilateral and contralateral CA1, S1 and contralateral striatum, subcellular morphology (TEM) for contralateral and potentially ipsilateral CA1, behaviour (NDS)
    - Hypothesis
        - We hypothesize that HTS-treated rats will display augmented cell shrinkage (i.e., decreased cell volume and increased density) in both hemispheres, and that this will result in increased subcellular damage. However, we hypothesize that despite this subcellular damage, we will still see improved behavioural outcome.
    - Timeline
        - All rats will receive 2 ten-minute handling sessions and an NDS training session prior to baseline.
        - 2 days prior to ICH, a NDS baseline will be performed.
        - On day 0, an ICH will be induced (3μL COL of 0.2U/μL in saline, or ~70μL bleed).[1]
        - HTS will be given via an IV bolus to the tail vein every 12 hours, starting at 2 hours post-stroke (i.e., 2 and 14 hours post-stroke).[2]
        - NDS will be assessed at 24-hours post-stroke.
    - Next steps

- If shrinkage is augmented and subcellular damage is observed, the experiment will first be replicated and may be examined with other timepoints.
    - Further experiments will then be conducted, if harm is still found. Blood flow impairment, functional consequences, cell death assays, and electrophysiology may be examined.
- If no shrinkage, subcellular damage, or benefit is observed, the study will conclude.

Statistics

- Experiment 1
    - Primary endpoint: Hematoma volume
    - Power: 80% power to detect a 25% change in hematoma volume, based on an expected volume of 80uL and SD of 14 as seen from Williamson[7].
        - N= 9 per group, increased to 12 in case of unexpected mortality.
    - Hematoma volume will be corrected against contralateral blood volume, and then analyzed using a two-tailed t-test.
    - Pain will be analyzed using independent samples two-tailed t-tests.
- Experiment 2
    - Primary Endpoint: Edema
    - Power: 80% power to detect a 1.5% absolute decrease in edema, based on an expected edema of 82% and SD of 1 as seen from Kung et. al and Williamson et al.[1,10]
        - n=17 per group, but increased to 20 in case of unexpected mortality.
        - Power is calculated for a 1% absolute change in edema, but if a 1% change is seen, this will be treated as biologically significant.
    - Edema in the ipsilateral striatum will be analyzed using two-way ANOVA with Tukey's HSD.
    - Mean ICP, if collected, will be analyzed using repeated measures ANOVA.
    - Cumulative probabilities of ICP change events, if collected, (peak ICP, magnitude of ICP spikes, ICP variability, and DIICP events) will be analyzed using Kolmogorov-Smirnov tests.[7,8]
    - Behaviour will be analyzed using a Mann-Whitney test.
- Experiment 3
    - Primary endpoint: Cell volume
    - Power: 80% power to detect a 15% difference in neuron soma volume, based on an expected volume of 2913 μm^3 and SD of 301 μm^3 as seen by Kalisvaart et al.[9]
        - N=9 per group, but increased to 12 in case of unexpected mortality.
    - Cell volume and density will be analyzed using a two-way ANOVA with Tukey's HSD.
    - Subcellular morphology assessed by TEM will be analyzed using a two-way ANOVA with Tukey's HSD.
    - Behaviour will be analyzed using a Mann-Whitney test.
    - Regression will be used to assess the relationship between relevant variables.

Relevant SOPs:

1. Anesthesia v3
2. Euthanasia v2
3. ICH v2
4. Stereological Assessment of Neurons in Vibratome Sections v2
5. EM sample processing
6. ICP telemetry implant v2
7. Neurological Deficit Scale - NDS
8. Edema Measurement

References

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Investigator Names, Signatures and Dates:

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