The Promise of Conditioning-Based Therapy for Aneurysmal Subarachnoid Hemorrhage

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SAH – High morbidity and mortality

- 30-day mortality 30-40%
- Severe disability 10-20%
- Cognitive dysfunction 50%
- Return to work 50%
Primary drivers of poor outcome – 1980’s

Aneurysm Rupture

- Initial Brain Injury
  - SAH
  - Global Ischemia
- Secondary Brain Injury
  - Vasospasm

Patient Outcome
Primary drivers of poor outcome – 2019

Aneurysm Rupture

Initial Brain Injury

SAH
Global Ischemia

Secondary Brain Injury

Early Brain Injury
(EBI)

Delayed Cerebral Ischemia
(DCI)

BBB breakdown
Cerebral Edema
Neuroinflammation
Neuronal cell death

Vasospasm
Microthrombi
Autoregulatory Dysfunction
Cortical Spreading Depression

Patient Outcome
Early Brain Injury (EBI)

- Occurs in 12% of patients
- Independent risk factor for poor outcome
- Time course: 1-3 days
- Etiology
  - BBB breakdown
  - Cerebral edema
  - Neuronal cell death
  - Neuroinflammation

Delayed cerebral ischemia (DCI)

- Occurs in 30% of SAH patients
- Independent risk factor for poor outcome
- Typical time course: 4-12 days
- Strongly associated with vasospasm
- However...
  - DCI occurs in absence of vasospasm
  - CONSCIOUS trials – ↓ vasospasm but no effect on outcome
- Other contributors to DCI
  - Microvascular dysfunction
  - Microvascular thrombosis
  - Cortical spreading depression
Endogenous Brain Protection

“What does not kill him, makes him stronger”
Friedrich Nietzsche
Preconditioning / Conditioning

• Exposure to sub-lethal quantities of a normally injurious stimulus can attenuate the severity of a subsequent injury and enhance reparative processes

Gidday, Nat Rev Neurosci, 2006
Preconditioning / Conditioning

- Numerous triggers
  - Hypoxia
  - Ischemia (global and focal)
  - Inflammation (e.g. LPS)
  - Epilepsy
  - Cortical spreading depression
  - Oxygen free radicals
  - Hypothermia and hyperthermia
  - CNS-specific antigens (MBP, MOG, E-selectin)
  - Pharmacological agents (deferoxamine, $K_{ATP}$ openers, inhalational anesthetics)

Preconditioning / Conditioning

Neuronal PC

Glial PC

Brain ischemic tolerance

Vascular PC

Gidday, *Nat Rev Neurosci*, 2006
Ways to translate PC to the clinic

“PC could be used as a therapeutic technique by inducing tolerance in individuals in whom ischaemic events are anticipated, such as high-risk surgical cohorts or patients with subarachnoid haemorrhage or transient ischemic attack.”
Conditioning-based therapeutics for SAH

- SAH = Ideal for conditioning-based therapy
  - High incidence of delayed brain injury after SAH
    - EBI – 12%
    - DCI – 30%
  - Potentially wide therapeutic window
    - EBI – Up to 1 day
    - DCI – Up to 4 days
  - EBI and DCI are multifactorial processes involving neurons, glia, and vessels → *Conditioning elicits a multifaceted protective response* in all of these cells
Conditioning-Based Therapy for DCI

Hypoxia
Endothelial Nitric Oxide Synthase Mediates Endogenous Protection Against Subarachnoid Hemorrhage-Induced Cerebral Vasospasm
Ananth K. Vellimana, Eric Milner, Tej D. Azad, Michael D. Harries, Meng-Liang Zhou, Jeffrey M. Gidday, Byung Hee Han and Gregory J. Zipfel

Stroke 2011;42;776-78
Hypoxic Pre-conditioning (PC) for DCI

Rotarod Pretraining

<table>
<thead>
<tr>
<th>Day -1</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 2</th>
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<tbody>
<tr>
<td>PC (8% O₂ for 4 hours)</td>
<td>Surgery (Sham or SAH)</td>
<td>Neuroscore and Rotarod</td>
<td>Vasospasm Assessment</td>
</tr>
</tbody>
</table>
Hypoxic PC prevents vasospasm after SAH
Hypoxic PC reduces neurological deficits after SAH
Hypoxic PC increases eNOS expression and activity
Hypoxic PC-induced DCI protection is eNOS-dependent
Hypoxic PC to prevent DCI

• Conclusions
  • Hypoxic PC provides powerful protection against DCI
  • Hypoxic PC-induced DCI protection is eNOS-mediated
Hypoxic Conditioning for DCI

• Unanswered Questions
  • Does post-SAH hypoxic conditioning provide similarly powerful DCI protection?
    • Important for clinical relevance

• What are the upstream molecular inducers of hypoxic conditioning-induced DCI protection?
  • Important for clinical translation
Does hypoxic post-conditioning (HPostC) provide DCI protection?
HPostC-induced DCI protection

1st Attempt – Standard hypoxic conditioning

- Rotarod Pretraining
- Neuroscore and Rotarod

Day -1
PC (8% O₂ for 4 hours)
Surgery (Pco or SAH)

Day 0
Day 1
Day 3
Vasospasm Assessment

Washington University School of Medicine in St. Louis
HPostC-induced DCI protection

2nd Attempt – Milder / repetitive hypoxic conditioning

Rotarod Pretraining

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<th>Neuroscore and Rotarod</th>
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Surgery (Sham or SAH)

Vasospasm Assessment

3hr 24hr 48hr

11% O₂ for 1hr  8% O₂ for 2hr
HPostC-induced protection against vasospasm
HPostC-induced protection against microthrombi
HPostC-induced protection against neuro deficits
Hypoxic Post-conditioning-induced DCI protection

• Conclusions
  • Hypoxic post-conditioning induces strong DCI protection
  • Therapeutic window may be narrower than anticipated → 3hr (at least in mice)
What are the upstream inducers of hypoxic conditioning-induced DCI protection?
Upstream inducers of hypoxia-induced DCI protection

• Expected characteristics
  • Hypoxia responsive
  • Regulator of eNOS expression / activity
Upstream inducers of hypoxia-induced DCI protection

- **Sirtuin1 (SIRT1)**
  - NAD-dependent protein deacetylase
  - Impacts broad range of physiological functions
    - Stress, inflammation, apoptosis, vessel function
  - May be upstream inducer of hypoxic PC-induced neurovascular protection in SAH
    - SIRT1 upregulated after hypoxia
    - SIRT1 implicated in PC-induced brain protection
    - SIRT1 upregulates eNOS expression and activity
Mouse studies
SIRT1 activity upregulated by HPostC
HPostC-induced vascular protection blocked by pharmacologic SIRT1 inhibition
HPostC-induced neurological protection blocked by *pharmacologic* SIRT1 inhibition.
HPostC-induced vascular protection blocked by genetic SIRT1 inhibition

Vasospasm

Microthrombi

% Area with microthrombi

0.0 0.5 1.0 1.5

SIRT1−/−: Sham: Normoxia
SIRT1−/−: SAH: Normoxia
SIRT1+/−: SAH: HPostC
HPostC-induced neurological protection blocked by genetic SIRT1 inhibition
*Genetic overexpression of SIRT1 mimics HPostC-induced DCI protection*

**A**

![Graph showing vessel diameter (µm) for Sham, SAH, WT, and Tg groups.](image)

**B**

![Graph showing neuroscore over days after SAH for WT Sham, WT SAH, Tg Sham, and Tg SAH groups.](image)
Pharmacologic activation of SIRT1 mimics HPostC-induced DCI protection
Functional Connectivity Study
Functional Connectivity

- Functional connectivity refers to the zero-lag Pearson correlation analysis performed across cortical regions.
- Functional connectivity can be measured via Optical Intrinsic Signal (OIS) imaging – a modality that measures correlations in vascular reactivity via quantitation of fluctuations in oxy- and deoxyhemoglobin in cortical brain regions of interest in mice.
Functional Connectivity

Primary motor (M1)
Secondary motor (M2)
Cingulate/retrosplenial (C/RS)
Primary sensory (S1)
Posterior retrosplenial (PRS)
Visual (V)

Kraft et al., PNAS, 2017
SAH induces deficits in functional connectivity

Largest effect on sensory and motor regions

Seed Maps
SAH induces deficits in functional connectivity
HPostC protects against SAH-induced deficits in functional connectivity via SIRT1
Rat Studies
Long-term neurobehavioral deficit study

- Male Wistar rats
- Pre-chiasmatic autologous blood injection (450 µl)
- Randomized to resveratrol (20 mg/kg ip bid for 7 days or vehicle)
- 33 day recovery
- Blinded measurement of neurologic/cognitive/histologic outcome
  - Neuroscore
  - Morris Water Maze neurobehavioral deficits
  - Neuronal cell loss
Resveratrol augments SIRT1 expression / activity

LD = 1 mg/kg
MD = 6 mg/kg
HD = 20 mg/kg
Resveratrol improves neurobehavioral outcome after SAH

Learning Set Morris Water Maze Performance
29-33 Days Post-SAH

P = 0.017
n = 13-14
Role of SIRT1 in HPostC-induced DCI protection

• Summary
  • HPostC augments SIRT1 activity
  • HPostC-induced DCI protection is blocked by pharmacologic and genetic inhibition of SIRT1
  • HPostC-induced DCI protection is mimicked by genetic overexpression and pharmacologic activation of SIRT1

• Conclusion
  • SIRT1 is a very promising new therapeutic target for DCI
Work in progress
What drives hypoxia-induced augmentation of SIRT1 activity?
Working hypothesis
NAMPT expression is increased after HPostC
HPostC-induced vascular protection blocked by pharmacologic NAMPT inhibition
HPostC-induced neurological protection blocked by pharmacologic NAMPT inhibition
Role of NAMPT in HPostC-induced DCI protection

• Summary
  • HPostC augments NAMPT expression
  • HPostC-induced DCI protection is blocked by pharmacologic inhibition of NAMPT

• Preliminary conclusion
  • NAMPT (and NAD\(^+\)) may also be promising new therapeutic targets against DCI
Conditioning-Based Therapy for DCI
 Isoflurane Conditioning for DCI

HIF-1α Mediates Isoflurane-Induced Vascular Protection in Subarachnoid Hemorrhage

Eric Milner¹,², Andrew W. Johnson¹, James W. Nelson¹, Michael D. Harries¹, Jeffrey M. Gidday¹,³, Byung Hee Han¹,³ & Gregory J. Zipfel¹,³,⁴

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Clinical evidence for conditioning-induced DCI protection
Preconditioning Effect on Cerebral Vasospasm in Patients With Aneurysmal Subarachnoid Hemorrhage

BACKGROUND: Recent experimental evidence indicates that endogenous mechanisms against cerebral vasospasm can be induced via preconditioning.

OBJECTIVE: To determine whether these vascular protective mechanisms are also present in vivo in humans with aneurysmal subarachnoid hemorrhage.

METHODS: A multicenter retrospective cohort of patients with aneurysmal subarachnoid hemorrhage was examined for ischemic preconditioning stimulus: preexisting steno-occlusive cerebrovascular disease (CVD) and/or previous cerebral infarct. Generalized estimating equation models were performed to determine the effect of the preconditioning stimulus on the primary end points of radiographic vasospasm, symptomatic vasospasm, and vasospasm-related delayed cerebral infarction and the secondary end point of discharge modified Rankin Scale score.

RESULTS: Of 1043 patients, 321 (31%) had preexisting CVD and 437 (42%) had radiographic vasospasm. Patients with preexisting CVD were less likely to develop radiographic vasospasm (odds ratio = 0.67; 95% confidence interval = 0.489-0.930; P = .02) but had no differences in other end points. In terms of the secondary end point, patients with preexisting CVD did not differ significantly from patients without preexisting CVD in mortality or unfavorable outcome in multivariate analyses, although patients with preexisting CVD were marginally more likely to die (P = .06).

CONCLUSION: This retrospective case-control study suggests that endogenous protective mechanisms against cerebral vasospasm—a preconditioning effect—may exist in humans, although these results could be the effect of atherosclerosis or some combination of preconditioning and atherosclerosis. Additional studies investigating the potential of preconditioning in aneurysmal subarachnoid hemorrhage are warranted.

KEY WORDS: Aneurysm, Preconditioning, Subarachnoid Hemorrhage, Vasospasm
Clinical evidence for conditioning-induced DCI protection

• Study design
  • Multicenter retrospective study
  • Patients with aneurysmal SAH
  • Presence of pre-existing cerebrovascular disease
    • $\geq 50\%$ stenosis of one or more extracranial or intracranial arteries supplying the brain
  
• Endpoints
  • Radiographic vasospasm
  • Symptomatic vasospasm
  • Vasospasm-induced infarction
  • Neurological outcome (mRS at discharge)  

Kim, Zipfel, et al., JNS, 2014
Logistic regression analysis for the predictors of vasospasm after SAH

<table>
<thead>
<tr>
<th></th>
<th>Pre-existing CVD (N=321)</th>
<th>No Pre-existing CVD (N=722)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radiographic Vasospasm</td>
<td>104 (32%)</td>
<td>333 (46%)</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>Symptomatic Vasospasm</td>
<td>65 (20%)</td>
<td>177 (25%)</td>
<td>P&lt;NS</td>
</tr>
</tbody>
</table>

Multivariate logistic regression analysis
OR=0.68 (95% CI= .489, .955, P<0.05)

Kim, Zipfel, et al., JNS, 2014
Evidence for a Conditioning Effect of Gas Anesthetics on Angiographic Vasospasm after Aneurysmal Subarachnoid Hemorrhage

Athiraman et al. (JNS, in review)
Clinical evidence for conditioning-induced DCI protection

• Study design
  • Single center retrospective study
  • Patients with aneurysmal SAH undergoing general anesthesia for aneurysm clipping or coiling
• Two anesthetic paradigms
  • Inhalational anesthetics only vs. Combined inhalational anesthetics + propofol
• Endpoints
  • Radiographic vasospasm
  • Symptomatic vasospasm
  • Neurological outcome (mRS at discharge)
Logistic regression analysis for the predictors of angiographic vasospasm after SAH

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Odds ratio</th>
<th>95% Confidence Interval</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhalational anesthetics</td>
<td>.35</td>
<td>.14-.89</td>
<td>.028</td>
</tr>
<tr>
<td>Hunt and Hess grade</td>
<td>1.51</td>
<td>1.03-2.22</td>
<td>.035</td>
</tr>
<tr>
<td>Diabetes</td>
<td>.19</td>
<td>.06-.55</td>
<td>.002</td>
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Logistic regression analysis for predictors of symptomatic vasospasm after SAH

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<th>95% Confidence Interval</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td>Desflurane (Average end-tidal concentration)</td>
<td>.415</td>
<td>.201-.859</td>
<td>.018</td>
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<tr>
<td>Modified Fisher</td>
<td>2.38</td>
<td>.986-5.75</td>
<td>.054</td>
</tr>
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</table>
Overall Conclusions
Overall conclusions

• Conditioning-based therapies have great promise for preventing / reducing brain injury after SAH
  • Strong experimental evidence (EBI and DCI)
  • Early human data encouraging (DCI)
  • Conditioning’s great advantage $\rightarrow$ targets *multiple* cell types and *multiple* injurious cascades
    • Neurons, glia, and vascular cells
    • EBI: BBB breakdown, neuroinflammation,
    • DCI: Large artery vasospasm, microvessel thrombi, and microvessel dysfunction
Aneurysm Rupture

Initial Brain Injury
- SAH
- Global Ischemia

Secondary Brain Injury

Early Brain Injury (EBI)
- BBB breakdown
- Cerebral Edema
- Neuroinflammation
- Neuronal cell death

Delayed Cerebral Ischemia (DCI)
- Vasospasm
- Microthrombi
- Autoregulatory Dysfunction
- Cortical Spreading Depression

Patient Outcome
Thanks

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