Decreased Acetylcholine in the Basal Forebrain: Insight to the Neurocognitive Deficits in the Subarachnoid Hemorrhage Patient

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OUTCOME FROM SUBARACHNOID HEMORRHAGE

- Good Recovery: 56%
- Moderate Disability: 18%
- Severe Disability: 7%
- Vegetative: 12%
- Dead: 4%
- Lost to Follow-Up: 3%

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Haley et al, J Neurosurg, 1993
Subarachnoid Hemorrhage

- Verbal Fluency
- Short/Long Term Memory
- Temporal Lobe Dysfunction
- “Global Amnestic Syndrome”
GOS ≠ QOL

- 1 year
  Excessive fatigue
  Lowered work status
- 4-7 year follow up
  41% memory problems (affecting ADL)
  35% daytime sleepiness
  20% reduced ability to work
  49% changed personality

Etiology?

- Blood products
- Subarachnoid Blood extravasation does NOT induce Neurocognitive alterations

Vascular Hypothesis

• Frontal lobe dysfunction resultant of focally decreased perfusion and or depressed metabolism...

• Vasospasm NOT indicator of deficit

Hillis et al J Neurol Neurosurg Psychiatry 2000;69:608-615
Positive Correlates

- Higher Fisher grade, frontal hematoma, intraventricular hemorrhage correlates with significantly worse neurocognitive outcome
  - Memory
  - Concentration
  - Attention
  - Perseveration

Cont’d

- Location of blood as well as amount of blood on CT correlated with memory dysfunction Acom’s show both long term and short term memory deficits

Larsson et al  Acta Neurol Scand 1994 Nov;90(5):331-6
Mavaddat et al  j Neurosurgery 1999 Sep;91(3) 402-7
Rationale: Deficits after ICH

- ICH found to cause deficits of higher cortical functioning
  - Short-term & long-term memory impairment from caudate ICH
    - Fuh et al. 1995
  - Deficits of higher-level perceptual functions
    - Su et al. 2000
  - Unilateral sensory neglect & constructional apraxia
    - Maeshima et al. 2002
Rationale: ACh-related deficits

- Acetylcholine implicated in learning and memory
  - In particular visuospatial discrimination tasks in marmosets treated with scopolamine (cholinergic-R blocking agent)
  - Ridley et al. 1984
- Impairment of memory in lesioned animals
  - Lesions to VDB (cholinergic basal forebrain projection to hippocampus) or NBM.
Alzheimer’s Dementia

- Recent/ Remote Memory
- Learning Memory
- Calculation
- Abstract Verbal Thinking
- Irritability/Concentration
Background-Rationale

- Basal Forebrain Cholinergic Complex: concentration of cholinergic neurons with multiple projections, esp hippocampus and cortex.
  - Nucleus basalis
  - Medial septal n.
  - Diagonal band n.
  - Substantia inominata
Background-Rationale

- In humans loss of cholinergic cells in n. basalis of Meynert seen with Alzheimer’s dementia.
  - Beginning of AD hallmarked by memory impairment
  - No cortical dysfunction (e.g. hemiparesis, sensory deficit, visual deterioration).
- Cholinergic loss implicated in cognitive dysfxn after TBI
Experimental Design - Immunohistochemistry

- Ab to C-terminus of vesicular acetylcholine transporter (VAT)
- Mark expression in perinuclear regions of soma & nerve terminals
- (VAT)-IR shown to be more sensitive, in cell body as well as axon terminal projections.
Methods

- 8 male Sprague Dawley Rats
  - 3 100µl injections in medial rostral forebrain
  - 3 10µl injections in lateral caudal forebrain
  - 2 saline control injections
Methods

- Focal injections of blood microinjected into the ventral forebrain
- Forty-micron thick tissue sections processed for immunoperoxidase localization of VAcht using the avidin biotin detection method
- Data analysis using acquisition of digital images using Image Pro Software
From: Butcher, Cholinergic Neurons and Networks
The Rat Nervous System, Paxinos ed., Academic Press
Results-VAT-IR

- >50% reduction in number of VAT-IR cells
- No variation cranial to caudal (44.7% and 47.4% of control respectively)
Vesicular Acetylcholine Transporter Labeling Following Arterial Blood Injections
Results-VAT-IR

Saline

ICH

# VAT-IR Cells

Saline

ICH
Experimental Design-Western Blot

• Confirmatory method to illustrate loss of VAT
  – presumed to represent cholinergic cell loss or at least loss of function of cholinergic cells.

• Rats sacrificed at 5 or 6 days post-op and sections of basal forebrain/VP dissolved in extraction buffer.
Results-Western Blot

- A: non-injected side;        B: injected side (ICH vs saline)

- Quantitative results pending
Conclusions

• Clinical correlation between hypocholinergic state of Alzheimers and subarachnoid hemorrhage patients “Global Amnestic Syndrome”

• Selective loss of acetylcholine in hemorrhage model with anatomic dependence
Phase II

- Larger cohort with dose/location variability
- Protective effects of Acetylcholinesterase inhibitors
- Recovery of basal forebrain loss after treatment with Acetylcholinesterase inhibitors
- Pre/Post cognitive testing with treatment in patients (IRB in progress)
Clinical Correlate (In Rats)

- Johnny and the Watermaze
Rationale: ICH pathology

• ICH results from rupture of small penetrating arteries.
  – ACA: medial lenticulostriates
  – MCA: lateral lenticulostriates
  – PCA: thalamogeniculates
  – Basilar: brainstem perforators

• EM studies: bleeding occurs at or near bifurcation of affected arteries, prominent degeneration of media smooth muscle seen.
Rationale: HTN #1 cause of ICH, HTN ICH in basal ganglia

- Hypertension (HTN) leading cause of ICH.
- Chronic HTN causes degenerative changes in vessel wall → reduced compliance, increase likelihood rupture.
- Basal Ganglia is most common site for hypertensive ICH
Summary

• ICH accounts for 10-20% strokes
• BG as frequent site of ICH (result of HTN)
• Deficits from ICH of higher cognitive function
  – Memory, perceptual functions and others
• ACh & basal forebrain cholinergic complex implicated in learning and memory
  – Especially visuospatial tasks
• Is there a connection???
Experimental Design

- Anatomy of rat brain
  - C-Pu
  - Ventral Pallidum ~ GPi
  - Basal nucleus
- Cholinergic basal forebrain complex very similar.
Experimental Design

- Blood extracted from femoral artery
- Stereotactic injection of 50-100 µL blood into ventral pallidum
- Rats sacrificed at 5 days
Experimental Design-Immunohistochemistry
Experimental Design-Western Blot

- Confirmatory method to illustrate loss of VAT
  - presumed to represent cholinergic cell loss or at least loss of function of cholinergic cells.
- Rats sacrificed at 5 or 6 days post-op and sections of basal forebrain/VP dissolved in extraction buffer.