Mechanisms of cellular and axonal injury in hydrocephalus: hypoxia-ischemia in slow motion

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Conflicts of Interest Disclaimer

- I served as a project consultant to Codman (1997-2001)
- I served as a project consultant to Medtronic (2002-2008)
Learning objectives: at the end of the talk the audience should understand:

• 1. Hydrocephalus causes gradual destruction of periventricular white matter axons through hypoxic, ischemic, and mechanical mechanisms
• 2. Metabolic disturbances likely contribute to reversible dysfunction
• 3. The clinical syndrome of hydrocephalic brain dysfunction is predominantly a subcortical disconnection syndrome.
Cerebrospinal Fluid (CSF)

• ~80% produced by choroid plexus, probably declines with age and some disease states

• ~20% water contributed by brain as product of glycolysis (NOTE: water moves freely across blood brain barrier)

• Absorption via arachnoid (villi) into venous system & via lymphatics associated with cranial and spinal nerves (relative contributions debated)

(see work by Miles Johnston)
Multiple functions of cerebrospinal fluid (CSF)

- Cleansing - wash away potentially noxious byproducts of metabolism
- Vehicle for molecular communication
- Protection / “cushioning” (NOTE: The brain does **NOT** float in CSF; density 1.036 vs. 1.006 g/cm³)
- Pressure damping - pulse wave from blood vessels is dissipated
Epidemiologic factors

Hospital admissions for shunt procedures
NIS database, US 2000, from Patwardhan et al. 2005
Pediatric causes – age 0-3 years
Adult “NPH” 70s-80s

Among pediatric cases (mean age 41 months) (from Massimi et al. 2009)

<table>
<thead>
<tr>
<th>Cause</th>
<th>1985-1990</th>
<th>2000-2005</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myelomeningocele</td>
<td>23%</td>
<td>15%</td>
</tr>
<tr>
<td>Aqueduct stenosis</td>
<td>6%</td>
<td>2%</td>
</tr>
<tr>
<td>Other malformation</td>
<td>20%</td>
<td>15%</td>
</tr>
<tr>
<td>Post hemorrhage</td>
<td>21%</td>
<td>22%</td>
</tr>
<tr>
<td>Tumor</td>
<td>20%</td>
<td>40%</td>
</tr>
<tr>
<td>Post infectious</td>
<td>10%</td>
<td>6%</td>
</tr>
<tr>
<td>Post trauma</td>
<td>2%</td>
<td>1%</td>
</tr>
</tbody>
</table>
What causes the ventricles to dilate?

• Considerable debate concerning the precise mechanics
• Obstructed CSF flow associated with increased CSF pulsatility
• DiRocco et al 1978, Communicating Hydrocephalus Induced by Mechanically Increased Amplitude of the Intraventricular Cerebrospinal Fluid Pressure (sheep, from 3cm to 9cm H$_2$O, 2 hours every 2 days)
Small forces applied repeatedly can very gradually distort the brain.

80mN ≈ 20mmHg
Same, but more gradual, effect with 20mN

Shulyakov, Del Bigio 2012 in press
Brain is a poroviscoelastic material

- Porous - fluid moves through extracellular spaces
- Viscous – material is deformed (strain) when a force (stress) is applied
- Elastic – tendency to return to original shape after the stress that caused the deformation is no longer applied
- Hakim 1971 made the “brain as a sponge” analogy – simple concept, complex mathematical & engineering principles
What happens after the ventricles enlarge?

• In human autopsy material, the main abnormality is atrophy / destruction of white matter structures.
Brain slices at autopsy

- Normal
- Moderate ventriculomegaly
- Asymptomatic hydrocephalus
- Severe ventriculomegaly
- Symptomatic hydrocephalus
HUMAN

Corpus callosum

Normal

Hydrocephalic

Alveus

Fornix

Anterior commissure

Del Bigio et al. 2003
• In human autopsy material, symptomatic hydrocephalus is associated with greater magnitude of white matter destruction.
• Shunting fails to restore the corpus callosum and fornix
• Animal model of hydrocephalus - kaolin (aluminum silicate) injection into cisterna magna (spreads into basal subarachnoid space)
• Acute inflammatory response and fibrosis (similar to post-meningsitis or post-hemorrhage)
• Feasible with almost all mammalian species at most ages
• Simple, inexpensive, reliable
• Dose dependent severity
Ventricles are enlarged, white matter is atrophic, and cortex is thin in the juvenile rat model of chronic hydrocephalus.
Magnetic resonance imaging shows severe enlargement of cerebral ventricles but only mild reduction of total brain volume.
Basic histopathological damage caused by hydrocephalus

- Enlargement of ventricles
- Loss of ependymal lining
- Destruction of periventricular axons
- Reactive glial changes (astroglial and microglial proliferation)
- Secondary changes in neurons
• Humans and experimental animals exhibit similar pathological changes due to hydrocephalus
• Therefore the animal models can be used to understand the pathogenesis of brain damage
Axon damage in corpus callosum – swollen axons shown with immunostaining to neurofilament and electron microscopy in young rats with kaolin-induced hydrocephalus.
Distant axon and neuronal changes

• Interruption of axonal / synaptic connections
• Secondary changes in dendritic structure - widespread
• Degeneration of long tracts
• Dying oligodendrocytes in corpus callosum of young rats with kaolin-induced hydrocephalus – necrotic and apoptotic patterns of nuclear debris
• Delayed myelination in immature brains (kittens Chumas 1994, young rats Del Bigio 1997, infants Hanlo 1997)
• Capillary density is reduced in periventricular brain tissue of adult rabbits with silicone oil-induced hydrocephalus, and is partially restored by shunting.
• Similar changes in H-Tx rats (Jones et al. 1991) and adult hydrocephalic dogs (Luciano et al. 2001)
Blood flow changes

• Cerebral blood flow and related parameters have been measured in human hydrocephalus using a variety of techniques including positron emission tomography ($^{15}$O-PET), transcranial Doppler, radionuclide angiography with Tc-99(m)-hexamethylpropylene amine oxime

• Children with hydrocephalus have decreased blood flow (Nishimaki 2004; Shirane 1992;)

• Adults with iNPH have reductions in regional cerebral blood flow (rCBF) (Brooks 1986; Chang 1999; Graff-Radford 1987; Klinge 2002; Kristensen 1996; Miyamoto 2007

• Improvement of blood flow after shunt correlates with clinical improvement in many studies
Magnetic resonance (arterial spin labeling perfusion) imaging shows reduced periventricular blood flow in hydrocephalic rats.

T2 images
Metabolic changes

- $[^{14}\text{C}]2$-deoxyglucose autoradiography in 1-month kittens with kaolin-induced hydrocephalus
- Frontal white matter has decreased blood flow and increased glucose utilization
- (Chumas 1994)
How does the metabolic change cause the oligodendroglial and axonal damage?
Ca\^{++}-mediated proteolysis contributes to axon damage (similar to acute ischemia and trauma)

Calpain content increased

Ca\^{++} accumulates

Suc-Leu-Tyr-MCA cleaved by calpain

Calpain cleavage products of spectrin in axons

Del Bigio, 2000
Consequences of calcium influx into damaged axons

Ca^{++}

− Phospholipase A2
− Membrane lipid damage
− μ Calpain activation
− Cytoskeleton damage
Possible explanation of calcium flux in damaged axons

- Sodium-calcium exchange pump
  - $\text{Ca}^{++}$ to $\text{Na}^{+}$

- $\text{Na}^{+}$-$\text{K}^{+}$ ATPase
  - $\text{Na}^{+}$ to $\text{K}^{+}$

- ATP
  - Ca++ ATPase

- Ca++ in endoplasmic reticulum

- Ca++ in L-type voltage sensitive calcium channels (VSCC) opened by depolarization

- Stretch-activated Na channels

- [Ca++]o = 1.2 µM
Pimonidazole traverses cell membranes in its oxidized form, and at $\text{PO}_2 < 10 \text{ mmHg}$, it forms irreversible covalent adducts with thiol groups in proteins, peptides, and amino acids.

Del Bigio, 2012
Increased 4-hydroxy-2-nonenol (marker of lipid peroxidation, red) in white matter of hydrocephalic rats is surrounded by reactive astrocytes (C), present in macrophages (D), and rarely colocalizes with GFAP (E,F) confirmed in quantitative biochemical assays.

Del Bigio, 2012
Nitric oxide (NO) can form peroxynitrite, which modifies amino acids to form nitrotyrosine (red). In white matter of hydrocephalic rats NT colocalizes with GFAP of astrocytes (C) and in microglia (Iba1, D).

Del Bigio et al 2012
Hypoxia-mediated brain damage in hydrocephalus

Del Bigio et al 2012
Summary – pathogenesis of white matter damage

• Hydrocephalic white matter is subject to decreased blood flow which in turn leads to hypoxic and nitrative stresses with minimal adaptive response

• Axons and oligodendrocytes are damaged
Shunting works if done before substantial axon damage occurs.
Hydrocephalus is a subcortical disconnection disorder.
Effects on the developing brain

- Retarded myelination (e.g. infants)
- Early onset (e.g. human fetal) can have adverse effects on the periventricular germinal tissue
- Potential consequence – altered brain development
In neonatal onset rat hydrocephalus, the subplate neuronal layer is disrupted and germinal matrix proliferation is reduced.
Kaolin-induced hydrocephalus in 14 day ferrets

At 35 days there is loss of subventricular zone cells and reduced cell proliferation (Ki67)

Di Curzio & Del Bigio 2012
Reduced Ki67 in ganglionic eminence of 21 week human fetus with hydrocephalus
What determines the damage?

- Age
- Rate of ventricle enlargement
- Intracranial pressure (cerebral perfusion pressure)
- Coexisting pathological changes (esp. cerebrovascular)
• There seems to be a threshold effect
• Ventricular enlargement is initially tolerated (if the intracranial pressure is not elevated)
Extracellular fluid movements

• Bulk flow occurs through interstitial spaces (0.1-0.3µl/min/g; Abbott 2004)

• Extracellular spaces ↑ white matter, ↓ gray matter (McLone 1971; Del Bigio 2008)

• Reduced flow of extracellular tracers in hydrocephalus (Shoesmith 1999)

• Waste products accumulate in CSF and brain tissue
Blood brain barrier in hydrocephalus

- Ultrastructural studies show subtle changes in endothelia of hydrocephalic brains
- Tracer studies using large molecules do not show increased permeability of the BBB in either direction
- Tracer studies using small molecules (<500 Da) has yielded inconsistent results
- Hydrocephalus in young rats is associated with random focal disruptions of small vessels (probably veins), but there is no generalized increase in BBB permeability
Are the changes reversible or preventable?

- Blood flow reductions and waste product accumulation are rapidly reversible by shunting.
- Axonal / neuronal function can recover if dysfunction is metabolic, however ...
- If axons are destroyed they cannot be replaced.
- Need to balance benefits of shunting with potential complications.
Hydrocephalus can be treated by redirecting CSF from the brain into the peritoneal cavity (or heart) with a “shunt”
Is a pharmacologic supplement needed for management of hydrocephalus?

• Shunt complications can be a major problem in small infants
• Decision to shunt not always easy in the elderly
• Pharmacologic protection might be useful in the period prior to definitive shunting
Potential targets for pharmacologic intervention

• Reduce CSF production (reviewed by Poca 2005)
• Enhance CSF flow / absorption
• “Neuroprotection” / axon protection
  – Reduce calcium influx
  – Reduce proteolysis axon cytoskeleton
  – Improve blood flow
  – Trophic support
• Improve recovery / regeneration post-shunt
Pharmacologic “neuroprotection” in hydrocephalic rats (Del Bigio et al.)

Control and treatment group stratified after MRI to ensure comparability

Blinded, vehicle control

Behavioral, imaging, histologic, and biochemical outcomes
Summary of Drug Treatments in Rats

- **Nimodipine** - Ca\(^{++}\) channel block, increased CBF; strong benefit behavior and structure (Del Bigio & Massicotte, 2001)
- **MgSO\(_4\)** - ? similar mechanism; mild benefit behavior, decreased astrogliosis (Khan et al. 2003)
- Nimodipine and MgSO\(_4\) in neonatal hydrocephalus (onset 2 days); toxic and without benefit - developmental differences in Ca\(^{++}\) handling and Ca\(^{++}\) channels (Khan et al. 2007)
- Mexiletine, riluzole - Na\(^{++}\) channel blockade (et al.); no benefit (Del Bigio et al. 2002)
- FK506, cyclosporin A, calpain inhibitor - axon protection via ?mitochondrial channels / neurophilins; no benefit (Khan et al. 2004)
- **Antioxidant diet** (analysis pending)
Lessons from the stroke literature

• To avoid waste of time and money, preclinical drug studies should include:
  – Long term behavioral and structural outcomes
  – Blinded evaluations
  – Proof of efficacy in animals with large brains more similar to humans (i.e. do initial testing in rodents but followup with another species)
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