Pathophysiology of intracerebral hemorrhage: Role of inflammation

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Disclosure

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- The use of endovascular devices for treatment of acute ischemic stroke is off-label use.
Intracerebral hemorrhage and inflammation

- Progressive disease?
- Forms of tissue injury?
- Experimental models?
- Summary?
- Immuno modulatory treatment?
Intracerebral hemorrhage and inflammation

- Progressive disease?
- Forms of tissue injury?
- Experimental models?
- Summary?
- Immuno modulatory treatment?
Intracerebral hemorrhage is a progressive disease
### Studies Evaluating Neurological Deterioration Following Intracerebral Hemorrhage

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients</th>
<th>Time interval</th>
<th>Definitions</th>
<th>Overall rates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Joon-Shik</td>
<td>122 patients</td>
<td>EMS-ER</td>
<td>&lt;2 GCS</td>
<td>18%</td>
</tr>
<tr>
<td>Qureshi</td>
<td>138 patients (GCS &gt; 8)</td>
<td>EMS-24 hours</td>
<td>&lt;2 GCS</td>
<td>33%</td>
</tr>
<tr>
<td>Mayer</td>
<td>46 patients (GCS &gt; 8)</td>
<td>During hospitalization</td>
<td>&lt;2 GCS</td>
<td>33%</td>
</tr>
<tr>
<td>Brott</td>
<td>103 patients (&lt; 3 hrs)</td>
<td>0-20 hours</td>
<td>&lt;2 GCS</td>
<td>48%</td>
</tr>
<tr>
<td>Leira</td>
<td>63 patients</td>
<td>0-48 hours</td>
<td>&lt;1 CSS</td>
<td>23%</td>
</tr>
<tr>
<td>Kazui</td>
<td>204 patients</td>
<td>During hospitalization</td>
<td>Consciousness or new deficit</td>
<td>25%</td>
</tr>
</tbody>
</table>
**Time Course of Neurological Deterioration**

Time interval between symptom onset and evaluation (days)

- **Joon-Shik**
- **Qureshi**
- **Mayer**
- **Brott**
Impact of Neurological Deterioration on Mortality After Intracerebral Hemorrhage

One month mortality

Mortality

ND | No ND
---|------

Mayer et al: Neurology 1994;1379-84

Three month mortality

Mortality

ND | No ND
---|------

Leira et al: Neurology 2004;461-67
Radiological Progression in Intracerebral Hemorrhage

Re: Qureshi AI, Mendelow AD, Hanley DF. Lancet. 373(9675):1632-44, 2009 May 9
Hematoma Enlargement


Baseline 6 hours
Hematoma Expansion (red) is the Major Cause of Neurological Deterioration

Other Cause(s) of Neurological Deterioration

Brott et al.
Kazui et al.
Mayer et al.
Leira et al.
Neurological deterioration represents an event that occurs during medical care and may be potentially preventable.

Neurological deterioration reflects clinically significant secondary processes.

Patients with neurological deterioration have a high mortality representing a high risk group.
Significance

- Neurological deterioration represents an event that occurs during medical care and may be potentially preventable.
- Neurological deterioration reflects clinically significant secondary processes.
- Patients with neurological deterioration have a high mortality representing a high risk group.

Significant secondary process = Inflammation?
Intracerebral hemorrhage and inflammation

- Progressive disease?
- Forms of tissue injury?
- Experimental models?
- Summary?
- Immuno modulatory treatment?
Pathophysiologic processes and inflammation

Pathophysiology of Intracerebral Hemorrhage

Hematoma

Edema
Surgical evacuation

- Decreased edema
- Decreased lactate
- Improved perfusion
rCBF Metabolism

Hibernation stage (0-2 days)  Reperfusion stage (2-14 days)  Normalization stage (>14 days)

Hypoperfusion without ischemia

Hypoperfusion without ischemia


Perihematoma ischemia never exists...
Metabolic failure is the primary event
Cell death of neurons and glia
Qureshi AI. Critical Care Medicine. 29(1):152-7, 2001

- Within hematoma
- Peri hematoma
- Distant to hematoma
Cell death of neurons and glia

Necrosis
Apoptosis
Blood-brain barrier disruption
Cell death of neurons and glia

A/B, representative CT slice before and after surgical evacuation of a lobar hematoma (within 24 h after symptom onset) of a lobar hematoma. The shrunken eosinophilic neurons necrosis (arrows), with minimal edema (X 200).

The TUNEL-positive nuclei of the apoptotic cells (long arrows)
Edema secondary to ICH may be more diffuse (global) than limited to the peri-hematoma region

- Recent evidence suggests that the secondary injury seen consequent to ICH is not limited to the peri-hematomal region.
- In experimental and clinical studies, expression of inflammatory markers, altered cellular water diffusion, hypoperfusion, and blood brain barrier breakdown have been detected in regions distant and contralateral to the hematoma.

Global Cerebral Edema among Good Grade Patients with Intracerebral Hemorrhage: Results from the Antihypertensive Treatment of Acute Cerebral Hemorrhage (ATACH) Study

- ATACH I is a multicenter prospective study recruited subjects with ICH and elevated systolic blood pressure (SBP) $\geq 170$ mm Hg who presented within 6 hours of symptom onset.

- The global cerebral edema or total brain volume change = Brain volume at 24 hrs -- Brain volume at baseline

Measurement of global cerebral edema

A. Initial CT scan image

B. Deletion of bone

C. Deletion of sulcal spaces (and interspace between brain surface and cranium)

D. Deletion of ventricles

E. Deletion of hematoma and peri-hematoma edema

Results

- A total of 18 (44%) of 41 patients had global cerebral edema.

- The median increase in brain volume among the 18 subjects was 35 cc ranging from 0.12 cc to 296 cc.

Cell death of neurons and glia
Qureshi AI. Critical Care Medicine. 29(1):152-7, 2001

Global effect

Within hematoma

Peri hematoma

Distant to hematoma
Intracerebral hemorrhage and inflammation

- Progressive disease?
- Forms of tissue injury?
- Experimental models?
- Summary?
- Immuno modulatory treatment?
Inflammation: Ischemic stroke

Tissue factor and adhesion molecules for leukocytes,
Release of interleukin-1 (IL-1), nitric oxide, factor VIII/von Willebrand factor, platelet-activating factor and endothelin,
Suppression of the thrombomodulin-protein C-protein S system,
Reduction of tissue-plasminogen activator and release of plasminogen activator inhibitor-1.

Tuttolomondo A. Curr Pharm Des. 2008;14(33):3574-89
Inflammation: ischemic stroke

- VASOACTIVE
- CYTOTOXIC
- THROMBOGENESIS

Tumor necrosis factor (TNF-alpha)
Interleukin (IL)-1
Interleukin (IL)-6

Kim KS. Lymphokine Cytokine Res 11: 293-298, 1992
Inflammatory mediators (source is neurons and glia **home grown**): Ischemic stroke

- Tissue factor and adhesion molecules for leukocytes,
- Release of interleukin-1 (IL-1), nitric oxide, factor VIII/von Willebrand factor, platelet-activating factor and endothelin,
- Suppression of the thrombomodulin-protein C-protein S system,
- Reduction of tissue-plasminogen activator and release of plasminogen activator inhibitor-1

Tuttolomondo A. Curr Pharm Des. 2008;14(33):3574-89
Experimental model of intracerebral hemorrhage

Regions for measurement of inflammatory markers

Tissue preparation and analysis- 1 hr after hematoma introduction

**HOMOGENIZED**
1% Nonylphenoxy polyethoxy ethanol, 100 mmol/L NaCl, 50 mmol/L Tris (hydroxymethyl) aminomethane hydrochloride, 2 mmol/L of ethylenediamine tetra-acetic acid containing leupeptin (100 [μg/ml]), aprotinin (100 [μg/ml]), 5 mmol/L 4-(2-aminoethyl) benzene sulfonyl fluoride.

**CENTRIFUGED**
20,000 xg for 20 minutes at 4[degrees]C and frozen at -70[degrees]C

**CYTOKINE ANALYSIS**
(IL-1[beta], IL-6, and TNF[alpha]) by the immunoassay method (Origen 1.5 Analyzer; IGEN, Inc., Gaithersburg, MD).
Tissue preparation and analysis- 1 hr after hematoma introduction

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CENTRIFUGED
20,000 xg for 20 minutes at 4[degrees]C and frozen at -70[degrees]C.

Concurrent serum and cerebrospinal fluid samples

CYTOKINE ANALYSIS
(IL-1[beta], IL-6, and TNF[alpha]) by the immunoassay method (Origen 1.5 Analyzer; IGEN, Inc., Gaithersburg, MD).
FIGURE - Mean arterial pressures over time in the intracerebral hemorrhage (ICH) and control groups.
## Regional measurements: TNF alpha

<table>
<thead>
<tr>
<th></th>
<th>ICH</th>
<th>Control</th>
<th>ICH</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zone 1</td>
<td>104.2±7.7</td>
<td>108.0±13.4</td>
<td>Zone 1</td>
<td>96.6±3.1</td>
</tr>
<tr>
<td>Zone 2</td>
<td>89.0±5.2</td>
<td>115.9±14.2</td>
<td>Zone 2</td>
<td>104.1±10.3</td>
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<tr>
<td>Cerebellum</td>
<td>95.6±6.5</td>
<td>94.4±3.8</td>
<td>Brainstem</td>
<td>90.3±4.4</td>
</tr>
<tr>
<td>Brainstem</td>
<td>90.3±4.4</td>
<td>119.0±11.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSF</td>
<td>7.1±1.3</td>
<td>10.8±2.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Regional measurements: Interleukin beta

<table>
<thead>
<tr>
<th></th>
<th>ICH</th>
<th>Control</th>
<th>ICH</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zone 1©</td>
<td>143.6±15.4</td>
<td>153.5±31.2</td>
<td>Zone 1</td>
<td>116.3±13.3</td>
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<tr>
<td>Zone 2©</td>
<td>120.2±9.1</td>
<td>121.3±26.7</td>
<td>Zone 2</td>
<td>139.1±15.9</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>169.8±19.1</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>143.6±15.4</td>
<td>153.5±31.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brainstem</td>
<td>120.2±9.1</td>
<td>121.3±26.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSF</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Serum measurements: TNF alpha

No evidence of pro-inflammatory cytokines in early period of ICH
Did we miss the surf: Sampled too early

Other injury models: rapid TNF[alpha] release is secondary to TNF[alpha] synthesis by cells in the central nervous system (including neurons and glia) rather than by migrating systemic inflammatory cells

Knoblach SM. J Neuroimmunol 95: 115-125, 1999
Inflammation and excitotoxicity: ischemic stroke

- **VASOACTIVE**
- **CYTOTOXIC**
- **THROMBOGENESIS**

Tumor necrosis factor (TNF-alpha)
Interleukin (IL)-1
Interleukin (IL)-6

Excitotoxic amino acids

Ischemia

Male and female New Zealand rabbits, each weighing 7 to 10 pounds,

Autologous arterial blood (0.4 mL)

Excitatory amino acids glutamate and aspartate and the inhibitory amino acid [gamma]-aminobutyric acid (GABA), using in vivo microdialysis for sampling extracellular fluid

Intra-parenchymal measurements: Glutamate (excitatory amino acid)

Intra-parenchymal measurements: Glutamate

ISCHEMIC STROKE
(Butcher SP, Stroke 1990; 21: 1727-1733)

Intra-parenchymal measurements: Glycine (inhibitory amino acid)

Acute mechanical compression: Intracellular release + impaired uptake

Acute mechanical compression: Intracellular release + impaired uptake

Excitotoxic amino acid release too short and small to invoke cytokine release
Intracerebral hemorrhage and inflammation

Progressive disease?

Forms of tissue injury?

Experimental models?

Summary?

Immuno modulatory treatment?
Neuronal and glial mechanical disruption; oligaemia/ischaemia

Glutamate release

Calcium influx, mitochondrial failure

Sodium accumulation, cytotoxic edema, necrosis

Calcium influx, mitochondrial failure

Sodium accumulation, cytotoxic edema, necrosis

Glutamate release

Calcium influx, mitochondrial failure

Sodium accumulation, cytotoxic edema, necrosis

Thrombin, ferrous iron, hemin, halo-transferrin release

Microglial activation

Oxygen free radicals

MMP

Complement factors

TNF-α

IL-1β

Cytochrome C

AQ-4 expression in astrocytes, breakdown of connective tissue in BBB, expression of adhesion molecules

Recruitment of PMNs and macrophages

BBB permeability increase, vasogenic edema

Mechanical

Cell membrane abnormalities

Secondary mediators

Re: Qureshi AI. Mendelow AD. Hanley DF. Lancet. 373(9675):1632-44, 2009 May 9
Neuronal and glial mechanical disruption; oligemia/ischemia

Calcium influx, mitochondrial failure

Glutamate release

Neuronal and glial mechanical stretch

Sodium accumulation, cytotoxic edema, necrosis

0-60 minutes

0-4 hours
Re: Qureshi AI, Mendelow AD, Hanley DF. Lancet. 373(9675):1632-44, 2009 May 9
4 hours-7 days
The pathway of inflammation

Thrombin, ferrous iron, hemin, halo-transferrin release

Microglial activation

- Oxygen free radicals
- MMP
- Complement factors
- TNF-α
- IL-1β

Caspase activation, apoptosis in neurons and glia

AQ-4 expression in astrocytes, breakdown of connective tissue in BBB, expression of adhesion molecules

BBB permeability increase, vasogenic edema

Recruitment of PMNs and macrophages

Recruitment of PMNs and macrophages

Recruitment of PMNs and macrophages

Recruitment of PMNs and macrophages
4 hours-7 days
Time course of inflammation vs neurological deterioration
CLINICAL PROGRESSION ALREADY OVER !!!!

- Thrombin,
- Ferrous iron,
- Hemin,
- Halo-
- Transferrin release

- Microglial activation,
- Caspase activation, apoptosis in neurons and glia
- AQ-4 expression in astrocytes, breakdown of connective tissue in BBB,
- Expression of adhesion molecules
- BBP permeability increase, vasogenic edema
- Recruitment of PMNs and macrophages
- Complement factors
- MMP
- Oxygen free radicals

- TNF-α
- IL-1β

Time course of inflammation vs neurological deterioration
4 hours-7 days
The pathway of inflammation

POST PARTY CLEAN UP

Thrombin, ferrous iron, hemin, halo-transferrin release

Microglial activation

Caspase activation, apoptosis in neurons and glia

TFNK-α expression in astrocytes, breakdown of connective tissue in BBB, expression of adhesion molecules

AQ-4 expression in astrocytes, breakdown of connective tissue in BBB, expression of adhesion molecules

BBB permeability increase, vasogenic edema

Recruitment of PMNs and macrophages

TNF-α, IL-1β

Complement factors

MMP

Oxygen free radicals

4 hours-7 days

POST PARTY CLEAN UP
Hematoma resorption: Prominent resorption within 7 d in 10% of patients

Day 0

Day 4

Re: Tariq N. International Stroke Conference 2009
Intracerebral hemorrhage and inflammation

Progressive disease?

Forms of tissue injury?

Experimental models?

Summary?

Immuno modulatory treatment?
Death at end of follow-up: Steroids versus control in patients with primary ICH

Death at end of follow-up: Steroids versus control in patients with primary ICH

Poor outcome at end of follow-up: Steroids versus control in patients with primary ICH

<table>
<thead>
<tr>
<th>Study or subgroup</th>
<th>Treatment n/N</th>
<th>Control n/N</th>
<th>Risk Ratio M-H,Fixed,95% CI</th>
<th>Weight</th>
<th>Risk Ratio M-H,Fixed,95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>At one month</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Desai 1998</td>
<td>10/12</td>
<td>12/14</td>
<td>17.3%</td>
<td>0.97</td>
<td>[0.70, 1.35]</td>
</tr>
<tr>
<td>Ogun 2001</td>
<td>14/15</td>
<td>11/12</td>
<td>19.1%</td>
<td>1.02</td>
<td>[0.82, 1.27]</td>
</tr>
<tr>
<td>Pounvarin 1987</td>
<td>37/46</td>
<td>41/47</td>
<td>63.5%</td>
<td>0.92</td>
<td>[0.77, 1.10]</td>
</tr>
<tr>
<td>Subtotal (95% CI)</td>
<td>73</td>
<td>73</td>
<td>100.0%</td>
<td>0.95</td>
<td>[0.83, 1.09]</td>
</tr>
<tr>
<td>Total events</td>
<td>61 (Treatment), 64 (Control)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterogeneity: Chi² = 0.52, df = 2 (P = 0.77); I² = 0.0%</td>
<td>Test for overall effect: Z = 0.76 (P = 0.44)</td>
<td></td>
<td></td>
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<tr>
<td>At six months</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subtotal (95% CI)</td>
<td>0</td>
<td>0</td>
<td>Not estimable</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>73</td>
<td>73</td>
<td>100.0%</td>
<td>0.95</td>
<td>[0.83, 1.09]</td>
</tr>
</tbody>
</table>

Poor outcome at end of follow-up: Steroids versus control in patients with primary ICH

Poor outcome at end of follow-up: Steroids versus control in patients with primary ICH

No benefit of steroid treatment in ICH pts

Conclusions

- The pathophysiology of intracerebral hemorrhage involves a primary effect and activation of secondary mediators.
- The role of inflammatory components as secondary mediators in pathophysiology of ICH is unclear.
  - Unlikely in acute neuronal/glial injury and therefore not a possible therapeutic target.
  - Possible role in late neural injury, cerebral edema, and hematoma resorption needs to be explored.
Zeenat Qureshi Institutes 2015—Thank you

St. Cloud, Minnesota, USA
Donka National Hosp, Conakry, Guinea

PingAn Hosp, Shijiazhuang, China
Xuan Wu Hosp, Beijing, China