The Blood-brain barrier breakdown as a therapeutic target in traumatic brain injury

Overview

1. Neurovascular unit
2. Traumatic brain injury (TBI)
3. Blood brain barrier (BBB) breakdown following TBI
   1. Pathophysiological progression
   2. Association with
      1. Seizure Development
      2. Neurodegeneration
4. Challenges for protecting the BBB
Neurovascular Unit

1) Functional unit of the blood brain barrier

2) Comprised of:
   1. Cerebral microvascular endothelium
   2. Astrocytes (including perivascular end feet)
   3. Pericytes (Perivascular macrophages)
   4. Extracellular Matrix (ECM)

Can include:
6. Microglia
7. Neuron components

(Ovalle et al., 2008)
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**Traumatic Brain Injury**

TBI is a form of “acquired brain injury” that “occurs when a sudden trauma causes damage to the brain”

- Associated with:
  - Change in consciousness
  - Permanent/ temporary change(s) in cognitive, physical, psychosocial functions

- Characterized by:
  - Time of onset (primary/secondary)
  - Location
  - Pattern of injury

TBI: Time Course

PUTATIVE CASCADE OF DAMAGING AND REPARATIVE EVENTS AFTER TBI

- Ionic alterations
- Excitotoxicity
- Free radicals
- Proteolytic mechanisms
- Inflammation
- Apoptosis
- Remodeling/plasticity

(Bradley, 2008)

Primary Brain Damage
Secondary Brain Damage
Blood Brain Barrier Dysfunction
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Blood Barrier Breakdown Following TBI

Main idea: pathophysiological changes increase permeability of the neurovascular unit

• Permeability changes:
  1. Tight junction dysfunction
  2. Altered pressure dynamics
  3. Altered membrane surface receptors
1. Permeability Changes: Tight junction dysfunction

- ↑ endothelium permeability via widened tight junctions

(Ivey et al., 2009)
1. Permeability Changes: Tight junction dysfunction

(Ivey et al., 2009)

JUST FOR INTEREST!
- Cytokines activate second messenger pathway
- Calcium is released and contracts actin filaments attached to the membrane bound tight junction components
- Tight junctions are pulled apart

Blood

Brain

Inflammation
1. Permeability Changes: Tight junction dysfunction

- Blood components move into intercellular space
  - Albumin
    - Most abundant plasma protein in humans
    - Functions to maintain osmotic pressure
    - Carrier of fatty acids, steroids and thyroid proteins
  - Other large molecular weight molecules (i.e. sugars, lipoproteins...etc.)

- Fluid and ions move into extra-cellular space
  - Water
  - $K^+$
1. Permeability Changes: Tight junction dysfunction

Both were injected with a large tagged fluorescent molecule which cannot normally traverse the membrane.

Note: Spread and leakage of tracer fluid in the TBI rat.

(Higashida et al., 2011)
2. Permeability Changes: Altered pressure dynamics

- The pressure difference between the artery and the surrounding tissue ↓
  - ↑ICP (intra-cranial pressure)
  - ↓cerebral perfusion and oxygenation

↑ Energy Demand  +  ↓ Energy Supply  =  ↑Increased neuronal death

Caused by immediate cytotoxic insult and excitatory release
Caused by decrease in oxygen and nutrient delivery
2. Permeability Changes: Altered pressure dynamics

Electron Micro-graphs of normal blood vessel in the rat

Electron Micro-graphs of blood vessel in the cerebellum of a rat exposed to a hypoxic insult

(Kaur et al., 2006)

Note: asterisks indicate Astrocytic feet
2. Permeability Changes: Altered pressure dynamics

Normal CT

Diffuse Neuronal injury following TBI

http://www.crash.lshtm.ac.uk/ctscanlarge.htm#caseone
Permeability Changes: Altered membrane surface receptors

A number of cell surface receptors on endothelial cells change their expression in response to inflammatory cytokines (Higashida et al., 2011)

- These molecules are responsible for:
  - Transporting other molecules across the membrane
  - Ie: glucose, insulin...etc.

(Abbott et al., 2006)
Permeability Changes: Altered membrane surface receptors

These molecules are also responsible for:
- Recruiting and facilitating the movement of immune cells across the endothelium into the neural space

(Rollins, 2001)
Permeability Changes: Altered membrane surface receptors

• Just for interest:
  • For more information on immune cell movement to site of injury

  • http://www.youtube.com/watch?v=WEGGMaRX8f0&feature=related
  • http://www.youtube.com/watch?v=ce0Xndms1bc&feature=related
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BBB Breakdown and Seizure Development

• Lasting abnormal rhythmogenesis associated with continued BBB disruption in patients after TBI (Korn et al., 2005; Hawkins & Davis, 2005)

– Evidence

A. Mannitol administration in CNS cancer patients preceded BBB breakdown and focal seizures (Marchi et al., 2007)

B. Serum albumin entry into the interstitial fluid increased with seizure activity (Seiffert et al., 2004; Cacheaux et al., 2007)
A. Mannitol Administration and Seizures

CNS lymphoma treatment in patients with and without Mannitol. Mannitol is a known BBB disruptor.

(Marchi et al., 2007)

### TABLE 1. Patient characteristics and summary of results

<table>
<thead>
<tr>
<th>ID #</th>
<th>Age</th>
<th>Sex</th>
<th>IAC only</th>
<th>Seizures after IAC</th>
<th>IAC + BBBD</th>
<th>Seizures after IAC + BBBD (yes/no)</th>
<th>Previous AED</th>
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<tr>
<td>1</td>
<td>57</td>
<td>F</td>
<td>0</td>
<td>0</td>
<td>17</td>
<td>2/15</td>
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<td>2</td>
<td>38</td>
<td>F</td>
<td>0</td>
<td>0</td>
<td>14</td>
<td>7/7</td>
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<td>3</td>
<td>72</td>
<td>M</td>
<td>3</td>
<td>0</td>
<td>11</td>
<td>7/4</td>
<td>Phenytoin</td>
</tr>
<tr>
<td>4</td>
<td>52</td>
<td>F</td>
<td>2</td>
<td>0</td>
<td>4</td>
<td>1/3</td>
<td>Phenytoin</td>
</tr>
<tr>
<td>5</td>
<td>33</td>
<td>F</td>
<td>2</td>
<td>0</td>
<td>7</td>
<td>4/3</td>
<td>Phenytoin</td>
</tr>
<tr>
<td>6</td>
<td>70</td>
<td>F</td>
<td>2</td>
<td>0</td>
<td>17</td>
<td>0/17</td>
<td>Phenytoin</td>
</tr>
<tr>
<td>7</td>
<td>20</td>
<td>F</td>
<td>2</td>
<td>0</td>
<td>20</td>
<td>3/17</td>
<td>Phenytoin carbamazepine</td>
</tr>
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<td>8</td>
<td>65</td>
<td>F</td>
<td>3</td>
<td>0</td>
<td>12</td>
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<tr>
<td>Total</td>
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<td></td>
<td></td>
<td>0</td>
<td>102</td>
<td>3.1*</td>
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<tr>
<td>Mean</td>
<td>50</td>
<td></td>
<td></td>
<td>0</td>
<td>±0</td>
<td>±0.9</td>
<td></td>
</tr>
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</table>

BBBD, blood–brain barrier disruption; IAC, intraarterial chemotherapy.
SEM indicates the standard error of the mean. The asterisk indicates a significant (p < 0.02) difference between seizure occurrence in the IAC versus BBBD groups.
See text for details.

Mannitol significantly increased seizure # compared to control

How are the seizures being generated?
B. Serum Albumin Uptake and Seizures

Recall: Serum albumin rapidly enters the neuronal microenvironment upon BBB dysfunction (Shlosber et al., 2010)

Findings from Ivens et al., (2007):
1. Following TBI in rats, albumin enters astrocytes

R: Albumin marker in control rat
L: Albumin marker in injured rat
B. Serum Albumin Uptake and Seizures

Findings from Ivens et al. (2007):

2. Albumin entry is mediated by TGF-β receptor (Transforming Growth Factor Beta)

R: Albumin marker in cortex (astrocytes) of injury exposed rat cortex.

L: Albumin marker in astrocytes of injured rats exposed to TGF-β receptor blocker
B. Serum Albumin Uptake and Seizures

Findings from Ivens et al., (2007):

3) Albumin uptake resulted in impaired ionic equilibrium leading to:
   
   a) transcriptional changes in K\(^+\) channels in astrocytes (graph not shown)
   
   b) seizure activity in prepared cortical slices

Top: Cortex cells (in vitro) displaying seizure activity, 1 week after exposure to albumin.

Bottom: Cortex cells (in vitro), displaying no seizure activity, 1 week after exposure to albumin + TGF-\(\beta\) receptor blockers.
B. Serum Albumin Uptake and Seizures

One model of seizure activity originating via BBB breakdown

(Friedman et al., 2009)
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BBB Breakdown and Neurodegeneration

• Sufficient evidence to conclude association between severe to moderate TBI and the development of dementia like Alzheimer’s symptoms (Bazarian et al., 2009)

– Evidence
  A. Accumulation of Amyloid- β (Aβ) in removed human cortex tissue after severe trauma (Ikonomovic et al., 2004)

  B. Trauma mimicked cellular environment alters Aβ transporters across in BBB (Yan et al., 2008)
A. Amyloid-β Accumulation After TBI

Aβ immuno-staining in excised human brain tissue compared to Alzheimer’s patient.

Note:
Difference in species, number of dense core plaques.

Amyloid-β immuno staining in excised human brain tissue at:
A: 2h and B: 16h

How did the Amyloid-β get there?  
(Ikonomovic et al., 2004)
B. BBB Aβ Transporter Changes Following Mock TBI Induction

- Two endothelial receptors are responsible for Amyloid-β transport
  - RAGE transports Aβ into the brain
  - LPR-1 transports Aβ out of the brain

(Yan et al., 2008)
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Protecting the BBB in TBI

• Challenges:
  1. No technology available to directly assess blood brain barrier pathology
     • Current techniques:
       – CT/MRI (contrast/non contrast images)
       – Indirect measure of plasma proteins in CSF (trauma situations)
  2. Therapies may not be selective for BBB
     – may have negative consequences for other vascular endothelium
  3. Time interval to treat is small
Protecting the BBB in TBI

• Challenges con’t:
  4. Proactive intervention affects seen in animal models may not translate to retroactive therapy affects seen in humans

4. Conflict of interest/bias in the research to reject the null hypothesis
  • Industry funded research (ie: pharmaceutical companies)

• Nevertheless one clinical trial of note:
  1. Pro-inflammatory peptide receptor blocker (Shakur et al., 2009)
Bradykinin and the BRAIN TRIAL:

• Bradykinin
  – Vasodilator
  – Has receptors on endothelial cells of BBB
  – Upon receptor binding (B2) it increases the permeability of the endothelial cells (Plesnila et al., 2001)
  – Blocking the bradykinin receptor shows a reduction in brain edema in rats after closed head injury (Ivashkova et al., 2006)

• The BRAIN TRIAL
  – human, international, multi-centered, randomized placebo controlled trial, using B2 receptor antagonist (Shakur et al., 2009)
Bradykinin and the BRAIN TRIAL:

- Bradykinin receptors located on endothelial cells and on astrocyte end-feet

(Abbott et al., 2006)
Bradykinin and the BRAIN TRIAL:

- Found no significant difference between between groups

### Table 1: Baseline Characteristics.

<table>
<thead>
<tr>
<th></th>
<th>High dose XY2405</th>
<th>Medium dose XY2405</th>
<th>Low dose XY2405</th>
<th>All doses XY2405 combined</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients randomised: n</td>
<td>57</td>
<td>56</td>
<td>58</td>
<td>171</td>
<td>57</td>
</tr>
<tr>
<td>Time since injury:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean (SD)</td>
<td>5.5 (1.7)</td>
<td>5.7 (1.9)</td>
<td>5.8 (1.8)***</td>
<td>5.7 (1.8)</td>
<td>5.9 (1.6)</td>
</tr>
<tr>
<td>median (min -- max)</td>
<td>5.8 (1.4 -- 8.0)</td>
<td>5.8 (2.1 -- 10.8)*</td>
<td>5.8 (1.8 -- 8.6)*</td>
<td>5.8 (1.4 -- 10.8)*</td>
<td>6.2 (2.3 -- 8.0)</td>
</tr>
<tr>
<td>≤ 1 hr (%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>&gt; 1hr - ≤ 3 hrs (%)</td>
<td>4 (7.0)</td>
<td>3 (5.4)</td>
<td>5 (8.6)</td>
<td>12 (7.0)</td>
<td>4 (7.0)</td>
</tr>
<tr>
<td>&gt; 3hrs - ≤ 8 hrs (%)</td>
<td>53 (93.0)</td>
<td>52 (92.9)</td>
<td>50 (86.2)</td>
<td>155 (90.6)</td>
<td>53 (93.0)</td>
</tr>
<tr>
<td>&gt; 8 hrs (%)</td>
<td>0</td>
<td>1 (1.8)</td>
<td>2 (3.4)</td>
<td>3 (1.8)</td>
<td>0</td>
</tr>
</tbody>
</table>

In animal studies B2 receptor blockers were administered PRIOR to head injury insult or less than 30 minutes later.

**Timing is everything!**
Protecting YOUR BBB

• Abstain from alcohol
  – ↑ Activation of ethanol metabolizing enzymes in BBB endothelial cells = ↑ in BBB breakdown

↑ tight junction disruption  ↑ Immune cell movement
Thank you

Questions?
References


