Neuroprotection for Stroke: Challenge of Crossing the Blood Brain Barrier

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Founded 1796
'the place of useful learning'
Ross Priory Loch Lomond
Formality
“The Mace”
Pure Scottish!
Objectives

• Discuss the mechanisms associated with cerebral ischemic-reperfusion and stroke
• Introduce novel strategies to mitigate ischemic reperfusion injury and stroke
• Discuss strategy for crossing the blood brain barrier
• Stimulate discussion on future clinical application of these strategies to stroke treatment
Disclosure

No financial disclosure, nor conflicts of interest

The novel experimental solutions presented are not FDA approved for clinical use and are considered to be experimental investigational.
Cerebral vascular events - sudden damage of brain induced by decreasing or suspending substrate delivery (oxygen and glucose) to the brain due to disturbances of brain vessels

Classification of cerebral vascular events (cerebral strokes)
1. focal cerebral ischemia (the most often – 80-88%)
2. intracerebral hemorrhage (9-15%)
3. subarachnoid hemorrhage (3-5%)

Normal values of cerebral blood flow
Cerebral blood flow (Q):
- cortex - 0.8 ml/g/min
- white matter - 0.2ml/g/min
Definitions of cerebral ischemia

It is the potentially reversible altered state of brain physiology and biochemistry that occurs when substrate delivery is cut off or substantially reduced by vascular stenosis or occlusion.

Stroke is defined as an “acute neurologic dysfunction of vascular origin with sudden (within seconds) or at least rapid (within hours) occurrence of symptoms and signs corresponding to the involvement of focal areas in the brain“ (Goldstein, Barnet et al, 1989)
Types of Stroke

- **Thrombosis**
  - Clot in intracranial carotid artery extends directly into middle cerebral artery

- **Emboli**
  - Clot fragment carried from heart or more proximal artery

- **Hypoxia**
  - Hypotension and poor perfusion cause border zone infarcts, no vascular occlusion

- **Subarachnoid hemorrhage**
  - Ruptured aneurysm

- **Intracerebral hemorrhage**
  - Hypertension
B. Pathogenetic mechanisms involved in development of cerebral ischemia (CI)

1. The brain is protected against focal interruption of blood supply by a number of extra- and intracranial collateral vessels.

Actual size of the cerebral ischemia depends on:

a) number and vascular tone of the leptomeningeal collateral channels

b) blood viscosity

c) blood perfusion pressure
The rich anastomotic connections between the carotid and vertebral arteries provide a powerful collateral system which is able to compensate for the occlusion of up to three of these arteries (known from animal experiment).

The good collateral system results in lesser ischemic area than is a territory supplied by occluded artery.

The bad collateral system results in ischemic area equal to a territory supplied by occluded artery.
Mechanisms involved in failure of collateral system

- ↓ systemic BP → ↓ blood flow through collateral circulation → base for hemodynamic theory of stroke development

- ↓ systemic BP + multifocal narrowing of extracerebral arteries → ↓ blood flow initially in the periphery of arterial territories

- since these regions represent the border lines between the supplying territories of the main cerebral arteries, the resulting lesion have been termed "border zone" or watershed infarcts
Ischemic cascade

Lack of oxygen supply to ischemic neurones

↓

ATP depletion

↓

Membrane ions system stops functioning

↓

Depolarisation of neurone

↓

Influx of calcium

↓

Release of neurotransmitters, including glutamate, activation of N-methyl-D-aspartate and other excitatory receptors at the membrane of neurones

↓

Further depolarisation of cells

↓

Further calcium influx

Carrol and Chataway, 2006
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Carrol and Chataway, 2006
Cosequences of brain ischemia

Energy failure / depolarisation

Transmitter release and receptor activation

Lipolysis (DAG \rightarrow PKC)

Protein phosphorylation

Proteolysis

Disaggregation of microtubuli

Enzyme conversion

Breakdown of cytoskeleton

Damage to membrane structure and function

Dysfunction of receptors and ion channels

Free radical formation

Inhibition of axonal transport, blebbing

Ca^{2+}
Carotid Occlusion
Open Carotid Endarterectomy
Percutaneous
Thrombolysis
Ischemic-Reperfusion
Potential Therapy
Pyruvate

- Natural carbohydrate and intermediary metabolite
- Energy-yielding fuel: bolsters cellular energy state ($\Delta G_{ATP}$)

Antioxidant:
Directly neutralizes $H_2O_2$, ·OH, ONOO⁻

Pyruvate → citrate → PFK → PP pathway → NADPH → GSH

Protocol: cardiac arrest - CPR

INFUSION: Pyruvate or NaCl

Pre-arrest  Arrest  CPR  ROSC

Time, min

Day 1  Day 2  Day 3

Defibrillate

NEUROLOGICAL EXAMINATION

BRAIN TISSUE SAMPLING

Terminal Biopsy
Neurological deficit day 2 post-arrest

H&E Staining of Hippocampus CA1

Sham

Arrest + NaCl

Arrest + Pyruvate
Neuronal Damage in Hippocampal CA1 subregion

Hippocampal Caspase-3 activity at 3h ROSC

*P<0.05 vs Sham; † P<0.05 vs Pyruvate

MMP-2 zymography

MMP-2 activity in the hippocampus 4h ROSC

T test, P = 0.006

Relative Density

CPR (n=4)    CPR+P (n=3)

CPR (n=4)    CPR+P (n=3)
Cerebellar Purkinje Cell 3d ROSC

Sham  
CPR  
CPR + P
Purkinje cell Number 3d ROSC

Purkinje cells 3d post cardiac arrest

ANOVA 1-factor, post-hoc Holm-Sidak method
Regulation of hypoxia-induced gene expression program

- HIF-1β
- HIF-1α
  - O₂, α-KG
  - Prolyl hydroxylase
  - HIF-1α-OH
  - VHL
  - HIF-1α-(Ub)^n
  - Ubiquitin
  - Proteosomal degradation

**Gene expression:**
- Erythropoietin (EPO)
- Heat shock protein-70 (Hsp-70)
- Endothelial NOS (eNOS)

**Cytoprotection**
Regulation of hypoxia-induced gene expression program

**Gene expression:**
- Erythropoietin (EPO)
- Heat shock protein-70 (Hsp-70)
- Endothelial NOS (eNOS)

**CYTOPROTECTION**
What if we could up-regulate EPO?
FOUNDATIONAL RESEARCH
Study: Pyruvate-enriched cardioplegia

- Yorkshire swine, 50-70 kg
- Groups: 4:1 blood:crystalloid cardioplegia (10/grp):
  - Control CPB (188 mM glucose)
  - Pyruvate CPB (glucose + 24 mM pyruvate)
  - Sham time controls (no cardioplegia, no CPB)
- Protocol: 60 min aortic crossclamp + hypothermic (20°C) cardioplegia, 30 min reperfusion on-pump, 4 h post-CPB recovery
- LV biopsy:
  - Energy metabolites, glutathione redox state (GSH/GSSG)
  - mRNA (RT-PCR): Erythropoietin (EPO), HIF-1α
  - Proteins (immunoblot): HIF-1α, EPO, EPO-R, Akt, P-Akt, Erk, P-Erk, eNOS, MMP-3, TIMP-2, CRP
Pyruvate cardioplegia increases myocardial content of EPO mRNA but not HIF-1α mRNA
Pyruvate cardioplegia enhances HIF-1\(\alpha\) and EPO protein contents
HIF-1α content correlates with EPO content in myocardium 4 h after cardiopulmonary bypass
Neutrophil marker enzyme myeloperoxidase in left ventricular myocardium

[Graph showing MPO, U/g tissue for Sham, Con, and Pyr conditions]
Myocardial water content 4 hours after CPB

- Sham
- Control
- Pyruvate

*P < 0.01 vs. sham
#P < 0.01 vs. pyruvate
Pro-inflammatory C-reactive protein (CRP) in LV myocardium 4 h after CPB
Gel zymography:
MMP-9

Relative MMP9 activity

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Control</th>
<th>Pyruvate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activity</td>
<td>1.00</td>
<td>1.40</td>
<td>0.60</td>
</tr>
</tbody>
</table>

* Significant difference
Ischemia-Reperfusion

Modified from Cherry et al. Age 2014. In press
Prospective Treatments

- **Erythropoietin**
  - Neuroprotective
    - Global Cerebral Ischemia
    - Stroke
    - Traumatic Brain Injury
    - Cerebral Hemorrhage
  - Only ~1% reaches the brain
  - Does not readily traverse BBB (34 kD; polyanionic)
  - Thromboembolism (Rabie & Marti 2008; Siren 2009)
Prospective Treatments

• Therapeutic Hypothermia
  • Targeted temperature management
  • Lower core body temperature during CPR
  • Lower metabolism → Less RONS (Dohi 2013)
  • Suppresses oxidative stress (Ostadal 2013)
  • Protects respiratory enzymes (Gong 2012)
• Only clinically effective treatment
• Requires intense training and monitoring
Prospective Treatments

- Ethyl Pyruvate
  - Electroneutral
  - Readily traverses BBB
  - Upregulates erythropoietin
  - Releases ethanol when cleaved by esterases
  - Poor water solubility
    - Maximal concentration: 1 mM

```
Ethyl Pyruvate: H₃C—C—C—O—CH₂—CH₃

esterases
H₂O

Pyruvate: H₃C—C—CO

Ethanol: HO—CH₂—CH₃
```
Prospective Treatments

So what do we give?
Pyruvate

- Natural carbohydrate
- Intermediary metabolite
- Energy-yielding fuel

http://commons.wikimedia.org/wiki/File:Pyruvate-3D-balls.png
Pyruvate

- Natural carbohydrate
- Intermediary metabolite
- Energy-yielding fuel

**PROCESS: OVERVIEW OF CELLULAR RESPIRATION**

1. **Glycolysis**
   - Glucose → Pyruvate
   - ATP
   - (two for every glucose)

2. **Pyruvate Processing**
   - Pyruvate → Acetyl CoA
   - CO₂

3. **Citric Acid Cycle**
   - Acetyl CoA → CO₂
   - NADH
   - FADH₂
   - ATP (or GTP)

4. **Electron Transport and Chemiosmosis**
   - Electron transport chain establishes proton gradient that is used to produce ATP
   - O₂ → H₂O
   - ATP
Pyruvate

- Natural carbohydrate
- Intermediary metabolite
- Energy-yielding fuel
- Antioxidant
  - Direct: \( \text{H}_2\text{O}_2 \cdot \text{OH} \ \text{ONOO}^- \)
  - Indirect: Pentose phosphate
    - ↑NADPH
    - ↑GSH/GSSG
  - Indirect: Erythropoietin

http://commons.wikimedia.org/wiki/File:Pyruvate-3D-balls.png
Pyruvate

- Pyruvate is more soluble than ethyl pyruvate
  - Doses may exceed 1 mM
  - Target concentration: 5 mM

- Pyruvate more rapidly neutralizes $\text{H}_2\text{O}_2$
  - By 1 h, almost completely

Pyruvate

Ischemia-Reperfusion

Pyruvate

ROS

Metabolic Enzyme Activity

Ca^{2+}

ATP production

GSH/GSSG

Pyruvate

eNOS Uncoupling

BH4

Lipid Peroxidation

Pyruvate

D_{2}

iNOS

NO

ONOO^{-}

Pyruvate

Macrophage Activation

Proinflammatory Signal Molecules

Immune Cell Recruitment

Cell Death

Bax/Bak

mPTP

Cyt C Release

Casp-9

Casp-3

Modified from Cherry et al. Age 2014. In press

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Experimental Model

1. Incision and Isolation of Vessels

Courtesy of Roger A. Hollrah, D.Chir., M.S.
Experimental Model
Experimental Model
Experimental Model
Specific Aim 1

[Diagram showing the metabolic pathways involving pyruvate, lactate, and the mitochondrial transport of potassium, sodium, and hydrogen ions.]
Specific Aim

• Hypothesis:
  • Pyruvate exerts antioxidant effects on brain during cardiac arrest-resuscitation, specifically by protecting activities of the enzyme components of the glutathione antioxidant system

• Rationale:
  • Previous studies in isolated hearts, open-chest models
  • Preserves metabolic machinery → ATP, NADPH
Specific Aim

A: Hippocampus

B: Cerebellum
Specific Aim

- GPx activity fell sharply after cardiac arrest
Specific Aim

- GPx activity fell sharply after cardiac arrest
- Pyruvate partially preserves activity in cerebellum
Specific Aim

- GPx activity fell sharply after cardiac arrest
- Pyruvate partially preserves activity in cerebellum
- Hyperoxic ventilation is sufficient to decrease activity

A: Hippocampus

B: Cerebellum
Specific Aim 2

- GRed activity fell sharply after cardiac arrest
Specific Aim 2

- GRed activity fell sharply after cardiac arrest
- Pyruvate had no effect
Specific Aim 2

- GRed activity fell sharply after cardiac arrest
- Pyruvate had no effect
- Enzyme activities fell in most groups as a function of time
  - Possible effects of hyperoxia
Specific Aim 2

- LDH activity is often assumed to be unaffected by RONS
- True in hippocampus at 1 h
Specific Aim 2

- LDH activity is often assumed to be unaffected by RONS
- True in hippocampus at 1 h
- Pyruvate increased activity in cerebellum
Specific Aim 2

- LDH activity is often assumed to be unaffected by RONS
  - True in hippocampus at 1 h
  - Pyruvate increased activity in cerebellum
- Time-dependent loss of LDH activity in all 3 groups
  - Hyperoxia?
Specific Aim 2

- Preliminary data suggest protective effect of pyruvate in cerebellum at 3 d ROSC
Specific Aim 2

- Cardiac arrest decreased GPx and GRed activity in hippocampus and cerebellum
  - Decreased LDH activity in hippocampus
- Cardiac arrest impairs main antioxidant defense
  - Pyruvate limited effect on glutathione system
- Pyruvate prevents damage, but not by the glutathione peroxidase-reductase system
Summary

- Pyruvate:
  - Promoted defibrillation to productive rhythm
  - Hastened glucose clearance
  - Improved redox state
  - Preserved cerebellar glutathione peroxidase activity
- Hyperoxia during resuscitation is a sufficient stimulus to impair glutathione peroxidase-reductase activity
Limitations

- Juvenile pigs
  - Free of disease
  - Compliant chest wall
  - Lean
- Animals are anesthetized during protocol
- Duration of pre-CPR arrest only 6 min
  - Maybe takes longer for EMS to respond
- Enzymes measured in tissue homogenates, not specific cells
ADDITIONAL STUDIES

• Upregulate Erythropoietin
Pyruvate minimizes rtPA toxicity from in vitro oxygen-glucose deprivation and reoxygenation
Myoung-Gwi Ryou, et al
Brain Research 14530; 2012: 66-75
Conclusions

• Pyruvate improves initial electrocardiographic and hemodynamic recovery from cardiac arrest
  • Decreased incidence of PEA upon cardioversion

• Pyruvate protects the brain from ischemia-reperfusion injury
  • Not by glutathione peroxidase-reductase system

• Time-dependent decrease in enzyme activities suggests that hyperoxia may have effects
Clinical Significance

- Pyruvate treatment is:
  - Natural
    - Every body makes it
  - Easy
    - Intravenous administration
    - No extra training requirement
  - Mobile
    - Stored easily
    - Crosses the BBB readily
  - Non-toxic
    - Safe within a wide range of concentrations
    - No obvious harmful side effects
Future Directions

• Impact of cardiac arrest and hyperoxia on:
  • Lung
  • Kidney
  • Liver

• Clinical trials
  • Cardiopulmonary bypass (Yurvati et al. 2003)

• Development of collaborative efforts with other fields
Future Directions

Cherry et al. Age 2014. In press
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