**Stroke**
Focal ischemic brain lesion
*Molecular Aspects of Neurological Diseases*

Philippe Lyrer
29.05.2007

**Aim**
- Close the gap!

**Overview**
- What is a stroke
- Animal models
- The ischemic cascade
- Translation to clinical trials
- Diffuse subcortical ischemic lesions
- Failure of clinical trials
- Outlook
Stroke - Definition

There are two main types of stroke

- **Ischemic stroke** 84%
  - is caused by blockage in an artery that supplies blood to the brain, resulting in a deficiency in blood flow (ischemia)
- **Hemorrhagic stroke** 16%
  - is caused by the bleeding of ruptured blood vessels (hemorrhage) in the brain
  - 10% intracerebral, 6% subarachnoidal.

Stroke – “biologic” Definition

During ischemic stroke, diminished blood flow initiates a series of events (called ischemic cascade) that may result in additional, delayed damage to brain cells.

Early medical intervention can halt this process and reduce the risk for irreversible complications.

Background Information

- 3rd most common cause of death
  - after heart attack and cancer
- Higher mortality with increasing age:
  - ≥ 60 years 2nd most common cause of death
- Men and women equally frequent
- Incidence: 150 - 200 / 100 000/year
  - Basel: 170 / 100 000/year (02-03)
  - 45-84 years old: 400 / 100 000/year
Ischemic brain lesion: cranial computer tomography early imaging (2 hours)

Dept. of Neuroradiology, University Hospital Basel, Switzerland

Ischemic brain lesion: delayed cranial computer tomography (48 hours)

Dept. of Neuroradiology, University Hospital Basel, Switzerland

Hemorrhagic brain lesion: Cranial Computer tomography

Exclude intracranial Hemorrhage!

Dept. of Neuroradiology, University Hospital Basel, Switzerland
Penumbra: DWI-PWI Mismatch

Diffusion / perfusion mismatch may be a marker for territory at risk.

Mechanism of cerebral ischemia

- cardiac Embolus 25-25%
- cerebral macroangiopathy 15-20%
- cerebral microangiopathy 30%

Embolic arterial occlusion

- Embolic clot occlusion of the middle cerebral artery (courtesy of A. Probst, Neuropathology Basel)
Animal models

- Rat: 4-Vessel occlusion (global)
  MCAO (focal)
- Mouse: MCAO (focal)
- Gerbil: CCAO (focal, global)
- Rabbit: clot model (t-PA model)
- Cat: cardiac arrest models
- Dog: cardiac arrest cardiac arrest
- Non-human primates: behavioral models

Note: Transient vs. Permanent vessel occlusion

MCA occlusion - permanent and temporary

Permanent group 30-min group 90-min group

Yasuki Ono, Shigehiro Morikawa, Toshiro Inubushi, Hiroaki Shimizu, and Takashi Yoshimoto

CT Schädel

Nativ  Spiral-CT mit KM

(Antwort: Neuroradiologie Universitätskliniken Basel, Prof. E. W. Radü)

PET from patient with acute MCA-occlusion

- Regional hypoperfusion at 3 hours after beginning of symptoms
- By reperfusion, no lesion on CT few days later
The penumbra concept


"Time is Brain"

CBF - Cerebral Blood Flow (ml/100ml/min)
- Gray matter 45-70 ml/100ml/min
- White Matter 20-24 ml/100ml/min

CBV - Cerebral Blood Volume (ml/100ml)
- Gray matter 4.7 %
- white matter 2-3%

MTT - Mean Transit Time (seconds)
- Mean time to traverse vasculature (NOT pixel)
- CBV:CBF ratio
What happens in stroke?

Blood supply drops suddenly threshold values:

Cerebral blood flow values:

- → 80-60 ml/min/100g: normal state
- → 37-60 ml/min/100g: protein syn., selective gene exp.
- → 20–36 ml/min/100g: lactic acidosis, cytotoxic edema
- → 10-20 ml/min/100g: energy deficit, glutamate tox.
- → 0-10 ml/min/100g: anoxic depolarisation, infarction

Cerebral blood flow

Magnetic resonance imaging

T2 DWI ADC

52 y/o WF with migraines, awoke with right H/A, L hemiparesis

(from Hakim; Neurology:51(supp3):S44-6)
Diagnostic Information in Acute Stroke: Diffusion Weighted Imaging

At 5 hours...

- Diagnostic Info: Assess initial infarct by DWI
- Prognostic Info: Predict infarct growth by PWI

After 5 days...

- Diagnostic Info: Assess initial infarct by DWI
- Prognostic Info: Predict infarct growth by PWI

Cytotoxic Edema: Diffusion Weighted Imaging

- Hindrance Restriction Barriers
- ATP-depletion
- Na⁺-K⁺ pump
- Cytotoxic edema
- Increased extracellular tortuosity
- Hindrance of water movement
- Diffusion weighted images appear bright

Penumbra: DWI-PWI Mismatch

- Patient with acute occlusion of A. cerebri media
**What happens in stroke?**

Consequences are disturbance:
- neurons, glial cell, astrocytes — simultaneously
- vessel lesion
- interstitial space
- secondary damages: edema, space occupation, recanalisation injury, bleeding

**Major pathways implicated in ischaemic cell death**

- excitotoxicity
- ionic imbalance
- oxidative and nitrosative stresses
- apoptotic-like mechanisms
- Disturbance of the neurovascular unit:
  - Proteolysis
  - inflammation


**Major pathways implicated in ischemic cell death**

Excitotoxicity and ionic imbalance:
the loss of energy stores results in ionic imbalance, neurotransmitter release and inhibition of respiration for e.g. glutamate, glutamate binds to ionotropes NMDA (N-methyl-D-aspartate) and AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptors promotes excessive calcium influx, which triggers an array of downstream phospholipases and proteases that degrade membranes and proteins that are essential for cellular integrity.

Mitochondria promotes oxygen radical generation and the release of death-inducing factors.
In addition, ionotropic glutamate receptors (GluRs) promote an excessive influx of sodium with concomitant cell swelling and ODEMA.

L-, P/Q- and N-type calcium channel functions mediate excessive calcium influx.
Intracellular, large amounts of zinc are stored in vesicles of excitatory synapses and are released upon depolarization.
1. At glutamatergic synapses, the action of glutamate is terminated by an efficient glutamate uptake system located in astrocytes.

2. Glutamate is cotransported with Na⁺, resulting in an increase in the intracellular concentration of Na⁺, leading to the activation of the Na⁺-K⁺-ATPase (2a). Glutamate is converted to glutamine by glutamine synthase (2b).

3. Activation of the Na⁺-K⁺-ATPase triggers aerobic glycolysis.
Glutamate toxicity - glucose

Lactate produced by the glutamate-stimulated glycolysis is released from astrocytes. A synaptic activation & direct glucose uptake into neurons under basal conditions.

Summary of glutamate-calcium release

- K⁺ efflux
- Excessive glutamate release and over excitation of glutamate receptors
- Ca²⁺ release from organelles
- Ca²⁺ influx and sequestration in the cell
- Intracellular accumulation of Ca²⁺ and Zn²⁺

- ends in activation of calmodulin dependent intracellular enzymes (phospholipases, endonucleases and protein kinases)

Major pathways implicated in ischemic cell death

Oxidative stress – radical generation

ROS reactive oxygen species

RNS reactive nitrogen species
Combination of superoxide (O$_2$–•) and nitric oxide (NO) generates the potent radical peroxynitrite anion (ONOO–•). Metal (Cu$^+$ and Fe$^{2+}$) catalysed pathways can also produce the hydrated radical (OH•) from hydrogen peroxide (H$_2$O$_2$).

**COX**, cyclooxygenase  
**GPX**, glutathione peroxidase  
**NOS**, nitric oxide synthase  
**SOD**, superoxide dismutase  
**XO**, xanthine oxidase

**apoptotic-like mechanisms Cell death pathways that are relevant to an apoptotic-like mechanism in cerebral ischaemia**

1. Cytochrome c release from the mitochondria is modulated by pro as well as anti-apoptotic Bcl2 family members. Cytochrome c release activates downstream caspases through apoptosome formation and caspase activation can be modulated by secondary mitochondria-derived activator of caspase (Smac/Diablo) indirectly through suppressing protein inhibitors of apoptosis (IAP).

**IAP**, inhibitors of apoptosis  
**PARP**, poly-(ADP ribose) polymerase  
**AIF**, Apoptosis inducing factor  
**Smac**, Secondary mitochondria derived activator of caspase  
**CAD**, caspase-activated deoxyribonuclease
2. Effector caspases (caspases 3 and 7) target several substrates, which dismantle the cell by clearing homeostatic, cytoskeletal, repair, metabolic and cell signaling proteins. Caspases also activate caspase-activated deoxyribonuclease (CAD) by cleavage of an inhibitor protein (ICAD).

3. Caspase-independent cell death might also be important. One mechanism proposes that poly-(ADP-ribose) polymerase (PARP) activation promotes the release of apoptosis-inducing factor (AIF), which translocates to the nucleus, binds to DNA and promotes cell death through a mechanism that awaits clarification.

References:
- Yang & Yankner, Nature 2000
Apoptosis

Yuang & Yankner, Nature 2000

Protease cascade involving members of the matrix metalloproteinase


Metalloproteinases

Yung et al. Nature Reviews 2001
Schematic view of the neurovascular unit or module, and some of its components


Cellular edema, irreversible cellular destruction, apoptosis via programmed metabolism due to proteases and endonucleases.

Generation of free radicals, arachidonic acid \(\rightarrow\) prostaglandin, oxidation of lipids, release of iron.

Activation of proteases, early gene expression, phospholipase A2 \(\rightarrow\) cell mediators: leukotriens, adhesion molecules, platelet aggregation factor.

Increase of intracellular Ca\(^{2+}\) due to influx of Ca\(^{2+}\) from extracellular space and release of Ca\(^{2+}\) from endoplasmatic reticulum and mitochondria.

Breakdown of the ATP-dependent Na-K-pump, loss of electric gradient, missing re-uptake and loss of glutamate, excitotoxicity.

Stop of metabolism, depletion of ATP, accumulation of lactate.

Overview of the ischemic cascade

Cause: Perfusion deficit, CBF <20ml/100g/min, complete, partial, time-dependent.

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Increase of intracellular Ca²⁺ due to inflow of Ca²⁺ from extracellular space and release of Ca²⁺ from endoplasmatic reticulum and mitochondria

Activation of proteases, early gene expression, phospholipase A₂ triggers production of arachidonic acid \( \rightarrow \) cell mediators: leukotriens, adhesion molecules, platelet aggregation factor

Generation of free radicals, arachidonic acid \( \rightarrow \) prostaglandin, oxidation of lipids, release of iron

Cellular edema, irreversible cellular destruction, apoptosis via programmed metabolism due to proteases and endonucleases
The concept of so-called neuroprotection

- Neuroprotective treatment
  - Glutamat-Antagonist
    - NMDA-Receptor-Antagonist (e.g. Magnesium, Lubeluzole)
    - AMPA-Receptor-Antagonist (e.g. Z-2000175)
    - Glyxin-Antagonist (e.g. GV-150526A)
  - GABA-Agonist (e.g. Clomethazol)
  - GABA-Analoga (e.g. Piracetam)
  - Calcium antagonist (e.g. Nimodipin)
  - Calcium channel blockers (e.g. BMS-204352)
  - Adenosin agonist (e.g. Acadesin, Propentofylfen, Pentoxifyllin)
  - S-HT\(_{1A}\)-Agonist (e.g. BAY X 3702)
  - Growth factors (e.g. Tielmerin)
  - Membrane-stabilizer (e.g. Cliazolin, Trilazaed)
- Anti-inflammatory treatment
  - Cytokin inhibitors (e.g. IL-1-Rezeptor-Antagonisten)
  - Immune modulation (e.g. Tacrolimus, Cyclosporin)
  - Free radical scavengers
  - Barbiturates


Treatment with thrombolysis and its therapeutic effect

| OR/NNT combined endpoints (mRS1, NIHSS 1, BI/95) | 0–90 min: OR 2.8; NNT-4 | 91–180 min: OR 1.5; NNT-9 |
| 181–270 min: OR 1.4; NNT-21 | 271–360 min: OR 1.2; NNT-45 |

From RCT:
- NINDS
- ECASS I + II
- ATLANTIS

Adjusted Odds ratio

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Brott et al., 2003

The concept of so-called neuroprotection

A. Richard Green, Tim Ashwood, Tomas Odergren, David M. Jackson, Pharmacology & Therapeutics 100 (2003) 195–214
Total of 71 identified trials
Mostly RCT
Result: no benefit at all
Compounds are safe to harmful

Neuroprotective clinical trials in UHBS

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<td>TEAST; 97-98</td>
<td>Trafermin (fibroblast growth factor)</td>
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SAINT-2 new trial

Investigational compound
Nitron, traps free radicals
i.v. therapy starts within 6 hours of stroke onset
Infarct size reduction in rat MCAO 62-69 %
What is wrong with neuroprotection?

"Is it not erroneous to lump together infarcts of all shapes, sizes, times, severities, and locations due to various occlusions (or no occlusions or site of occlusion unknown) and trust the statisticians to make sense of it all through randomization into underpowered trials?"


Reasons for neuroprotectants failure

- Pharmacologic reasons
  - pharmacologic target not relevant to humans
  - wrong dose
  - wrong treatment duration
  - unsuitable pharmacokinetics
- Unsuitable study design
  - Use of wrong measurements (outcomes)
  - delayed treatment
- Statistical Power
  - overestimation of therapeutic effect (>10%)
  - underestimation of clinical relevant effects
- Pathophysiological heterogeneity of the disease
  - no biological substrate to salvage
  - no penumbra
  - lack of reperfusion


Future design for neuroprotective trials

More standardized stroke syndromes for inclusion in trials
Animal models should be standardized
Short time window
Salvageable tissue in the DWI/PWI MRI
Molecule that passes BBB
Co-administration of thrombolytic therapy
Leukoaraiosis

RE030736, m 66-years, no hypertension

JJ160139, m 61 yrs

Hypertension

Subcortical hypertensive arteriosclerotic encephalopathy

JJ160139, m 61 yrs, hypertension

Pathology – microscopic appearance of affected vessels (arterioles)

Lipo-hyalinosis

Thrombosis

Virchow-Robin Space

from A. Probst, Neuropathology, Basel
Vascular Dementia - Epidemiology

- Prevalence (%) in Europe/USA:
  1989 Boston   AD  8.7  VaD  0.9
  1990 London   3.1  0.1
  1991 Stockholm 6.0  3.0
  1995 Rotterdam 4.5  1.0
  1997 Odense   4.7  1.3
- Ratio AD/VaD: <0.1-0.5!

Review, Acta Psych Scand 2001;104:4-11

Blood pressure and risk of stroke

Per 5-6mmHg diastolic or 10-12 systolic BP-elevation: 38% relative Risk increase
Animal models: Genetically predisposed rats

Spontaneously Hypertensive rats (SHR)

Origin:
Chamoto at the Kyoto School of Medicine in 1963 from an outbred Wistar Kyoto male with marked elevation of blood pressure mated to female with slightly elevated blood pressure.

Characteristics:
- Hypertension, insulin resistance, hyperinsulinemia, hyperglycemia, hypercholesterolemia.

Spontaneously Hypertensive Stroke Prone rats (SHR-SP)

Characteristics:
- 82% of males will develop cerebrovascular lesions (cerebral hemorrhage or infarction) over 100 days of age.
- Hypertension, nephropathy, insulin resistance, hyperinsulinemia, hyperglycemia, hypercholesterolemia.

Anatomical abnormalities:

- Variable branching pattern of the distal MCA.
- Morphological arrangement of vascular smooth muscle cells are disorganized in the basilar artery of the SHR-SP, influence collateral circulation, morphological changes of blood flow, or vulnerability of the arterial wall to high blood pressure.
- Abnormalities in the BBB: plasma components leakage through arteriols: induce fibrinoid necrosis of small arteries, severe brain edema and lacunar infarction.
- No atherosomatous lesions.

Vascular physiology:
- Response of the cerebral artery to substances causing endothel-dependent vasodilatation impaired in SHR-SP.

Genetic loci involved:
- Chromosomes 1 and 18: genes involved in blood pressure.
- Chromosome 5: blood pressure independent, co-localized with genes encoding atrial and brain natriuretic factor.

Blood pressure according to different strains

Hypothetical hypertension genes of SHR and SHR-SP

Nabika T. et al., Cellular and Molecular Neurobiology 2004;24:639-646

Vascular permeability in WKY, SHR and SHR-SP


Differences between SHR and SHR-SP strains

Differences between Wistar and stroke prone SHR

Smooth muscle actin staining, increased in SHR-SP with hypertrophic arteries (Anterior cerebral artery)

Klüver-Barrera staining of white matter in corpus callosum

Appearance of white matter lesions in the brain of SHR, SHR-SP

Col4 and GFAP in normotensive and hypertensive rats

**Fraction GFAP-positive (in %)**

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**Density of Collagen 4-positive vessels**

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**Fraction Collagen 4-positive (in %)**

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