Stroke
Focal ischemic brain lesion
Molecular Aspects of Neurological Diseases

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Aim

• Close the gap!

Clinician  Scientist
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 25-35%
- Cerebral macroangiopathy 15-20%
- Cerebral microangiopathy 30%

© M. Mumenthaler, H. Mattle; Thieme Verlag, Stuttgart, 1997

Embolic Artrial 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*


MCAO: Three-vessel occlusion using a micro-clip for the proximal left middle cerebral artery

Lit.: Hiroji Yanamoto, Izumi Nagata, Nobuo Hashimoto and Haruhiko Kikuchi
MCA occlusion – permanent
(autoradiography)


MCA occlusion - permanent and temporary

Yasuki Ono, Shigehiro Morikawa, Toshiro Inubushi, Hiroaki Shimizu, and Takashi Yoshimoto
Mouse model of transient cerebral ischemia – temporary MCAO with coated nylon filament


CT Schädel

Nativ

Spiral-CT mit KM

(Aufnahme: 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

Perfusion Basics

- 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

(from Hakim; Neurology:51(supp3):S44-6)
52 y/o WF with migraines, awoke with right H/A, L hemiparesis

**Diagnostic Information in Acute Stroke: Diffusion Weighted Imaging**

At 5 hours...

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

Lo H., et al. nature rev. 2003
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 reuptake for e.g. glutamate, glutamate binds to ionotropic 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.
Major pathways implicated in ischemic cell death

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 OEDEMA.

RNS reactive nitrogen species

ROS reactive oxygen species

Ionic imbalance -Calcium metabolism

L, P/Q and N-type calcium channel functions mediate excessive calcium influx. Besides calcium, large amounts of zinc are stored in vesicles of excitatory neurons and are co-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.

4. Lactate produced by the glutamate-stimulated glycolysis is released from astrocytes. A, synaptic activation; B, direct glucose uptake into neurons under basal conditions.
Summary of glutamate-calcium release

- $K^+$ efflux
- Excessive glutamate release and over excitation of glutamate receptors
- $Ca^{2+}$ release from organelles
- $Ca^{2+}$ influx and sequestration in the cell
- Intracellular accumulation of $Ca^{2+}$ and $Zn^{2+}$

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

Major pathways implicated in ischemic cell death

Generation of oxygen and nitrogen radicals

Combination of superoxide (O2–•) and nitric oxide (NO) generates the potent radical peroxynitrite anion (ONOO–•). Metal (Cu+ and Fe2+) catalysed pathways can also produce the hydroxyl radical (OH•) from hydrogen peroxide (H2O2).

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

IAP inhibitors of apoptosis
PARP poly-(ADP ribose) polymerase
AIF Apoptosis inducing factor

Smac Secondary mitochondria derived activator of caspase
CAD caspase-activated deoxyribonuclease

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).

2. Effector caspases (caspases 3 and 7) target several substrates, which dismantle the cell by cleaving homeostatic, cytoskeletal, repair, metabolic and cell signalling proteins. Caspases also activate caspase-activated deoxyribonuclease (CAD) by cleavage of an inhibitor protein (ICAD).
Apoptotic-like mechanisms: Cell death pathways that are relevant to an apoptotic-like mechanism in cerebral ischaemia.

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.

Yuang & Yankner, Nature 2000
Apoptosis

Central role of mitochondria in neuronal apoptosis

IAP: inhibitor of apoptosis proteins

Protease cascade involving members of the matrix metalloproteinase


Metalloproteinases

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


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

Stop of metabolism, depletion of ATP, accumulation of lactate

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

Increase of intracellular Ca$^{2+}$ due to inflow of Ca$^{2+}$ from extracellular space and release of Ca$^{2+}$ from endoplasmatic reticulum and mitochondria

Activation of proteases, early gene expression, phospholipidase 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
Overview of the ischemic cascade

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

Stop of metabolism, depletion of ATP, accumulation of lactate
Overview of the ischemic cascade

Cause: Perfusion deficit, CBF, partial, complete, time-dependent.

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

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

Increase of intracellular Ca\(^{2+}\) due to inflow of Ca\(^{2+}\) from extracellular space and release of Ca\(^{2+}\) from endoplasmatic reticulum and mitochondria.
Overview of the ischemic cascade

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

- Stop of metabolism, depletion of ATP, accumulation of lactate
- Breakdown of the Na-K-pump, loss of electric gradient, missing reuptake and loss of glutamate, excitotoxicity
- Increase of intracellular Ca$^{2+}$ due to influx of Ca$^{2+}$ ions

**Activation of proteases,** early gene expression, phospholipase A2 triggers production of arachidonic acid $\rightarrow$ cell mediators: leucotriens, adhesion molecules, platelet aggregation factor

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Overview of the ischemic cascade

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

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

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

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

5. **Activation of proteases, early gene expression, phospholipase A2 triggers production of arachidonic acid / cell mediators: leucotriens, adhesion molecules, platelet aggregation factor**

6. **Generation of free radicals, arachidonic acid → prostaglandin, oxidation of lipids, release of iron**

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

Dynamics in cerebral ischemia - Schematic overview of dynamics in neuronal ischemic cell death

The concept of so-called neuroprotection

- Neuroprotective treatment
  - Glutamat-Antagonist
    - NMDA-Receptor-Antagonist (e.g. Magnesium, Lubeluzole)
    - AMPA-Receptor-Antagonist (e.g. ZK200775)
    - Glycin-Antagonist (e.g. GV-150526A)
  - GABA-Agonist (e.g. Clomethiazol)
  - GABA-Analoga (e.g. Pirazetam)
  - Calcium antagonist (e.g. Nimodipin)
  - calcium channel blockers (e.g. BMS-204352)
  - Adenosin agonist (e.g. Acadesin, Propentofyllin, Pentoxifyllin)
  - 5-HT₁A -Agonist (e.g. Ipsapiron, BAY X 3782)
  - growth factors (e.g. Trafermin)
  - Membrane-stabilizer (e.g. Citicholin, Tirilazad)
- 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, BI³95)
0–90 min: OR 2,8; NNT=4
181-270 min: OR 1,4; NNT=21
271-360 min: OR 1,2; NNT=45

OR=Odds ratio
(adj = ratio of number with/without event)

From RCT:
NINDS
ECASS I + II
ATLANTIS

Brott et al., 2003
The concept of so-called neuroprotection

Total of 71 identified trials
 Mostly RCT
 Result: no benefit at all
 Compounds are safe to harmful

A. Richard Green, Tim Ashwood, Tomas Odergren, David M. Jackson; Pharmacology & Therapeutics 100 (2003) 195–214
### Neuroprotective clinical trials in UHBS

<table>
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<tr>
<th>N patients</th>
<th>Trial; years</th>
<th>Drug</th>
<th>Result</th>
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<td>TESS; 94-95</td>
<td>Tirilazad</td>
<td>Negative - harmful</td>
</tr>
<tr>
<td>4</td>
<td>TEAST; 97-98</td>
<td>Trafermin (fibroblast growth factor)</td>
<td>harmful</td>
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<tr>
<td>20</td>
<td>POST 010; 00-01</td>
<td>BMS 204352</td>
<td>Negative safe</td>
</tr>
</tbody>
</table>

### SAINT-2 a recent 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**¹
  - overestimation of therapeutic effect (>10%) 
  - underestimation of clinical relevant effects

- **Pathophysiologic heterogeneity² 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

Leucoaraiosis

JJ160139, m 61 yrs
Hypertension

RE030736, m 66-years,
no hypertension
Subcortical hypertensive arteriosclerotic enzephalopathy

JJ160139, m 61 yrs, hypertension

Pathology – microscopic appearance of affected vessels (arterioles)

from A. Probst, Neuropathology, Basel

Lipo-hyalinosis

Virchow-Robin-Space

Thrombosis

+ Fibrinoid necrosis
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)

Origine:
Okamoto 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, hypertriglyceridemia, 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, hypertriglyceridemia, hypercholesterolemia.
Animal models: Genetically predisposed rats

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, rheological 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 atheromatous 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

- Hypertension genes + ‘extra’ Hypertension genes + Stroke-susceptibility genes
- Hypertensive Stroke resistant
- More hypertensive Stroke-prone

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

Vascular permeability in WKY, SHR and SHR-SP

- Horse-radish peroxidase accumulation

Collagen staining reveals significant differences in the distribution of collagen in MCA from SHR-SP compared with SHR.

**Differences between SHR and SHR-SP strains**


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

**Differences between Wistar and stroke prone SHR**

**Klüver-Barrera staining of white matter in corpus callosum**

Disturbed structure of the white matter in CC from SHR-SP: disarrangement of nerve fibers, marked vacuoles and disappearance of myelinated fibers.


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

- Normal (grade 0)
- Disarrangement of the nerve fibers (grade 1)
- Formation of marked vacuoles (grade 2)
- Disappearance of myelinated fibers (grade 3)

Col4 and GFAP in normotensive and hypertensive rats

The End