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Stroke

Hallmark is **sudden** loss of neurological function

Two principle pathological processes:

Cerebral ischaemia or infarction- occlusion of arteries supplying blood to brain

- Wide range of severity

2. Spontaneous intracranial haemorrhage

- This is by far the most destructive, higher mortality rate and higher incidence of severe neurological deficit but is less common
Pathogenesis

- Critical reduction in blood flow
- Reduction in arterial oxygen
- Arterial thrombosis
- Embolic arterial occlusion
- Head injury
- Subarachnoid haemorrhage
Causes of acute stroke

- Infarction
  - Atheroma

Thrombus (Clot)
Cerebrovascular disease

- Hypoxic damage occurs due to reduction in blood supply to CNS
- Cells most vulnerable are neurons due to almost exclusive dependency on oxidative glucose metabolism
- Ischaemic neuronal death occurs with 5-7 mins and is characterised by activation of glutamate receptors, causing uncontrolled entry of Ca
- Early stages are reversible, but final stages are not
**Gross Pathology**

**Penumbra** = the volume of the brain tissue that suffers ischaemia, but in which the ischaemic damage is potentially reversible. Mediators expressed in penumbra include cytokines and proteases which contribute to cell death.

**Core** = focal irreversibly damaged area.
Inflammatory reaction in stroke

- Leukocytes accumulate in the ischaemic area, particularly neutrophils.
- Macrophages and microglia accumulate in the affected area up to several days post stroke, they release cytokines and cause neuronal damage. This leads to:
  - Obstruction of capillaries
  - BBB damage
  - Vasoconstriction
  - Release of cytotoxic mediators

Results in damage to neurons in penumbra
Putative cascade of damaging events in focal cerebral ischaemia

Dirnagl et al 1999, TINS
Increase in leukocyte traffic leads to:

- Impaired blood flow
- Release of reactive oxygen intermediates and matrix metalloproteinases
- Release of prostaglandins and platelet activating factor which leads to vasoconstriction and platelet aggregation
- Local cytokine production leads to neuronal damage
ADAMTSs

- ADAMTS-1, -4 and -5 are secreted protease enzymes, which bind to the ECM and degrade it
- ADAMTS-1 inhibits VEGF induced angiogenesis
- Aggrecanases inhibited by: TIMP-3 and catechin gallate esters
Metzincins
basic domain organisation

- MMP
- ADAM
- ADAMTS

- Signal Peptide
- Prodomain
- Catalytic Domain
- Furin recognition site
- Disintegrin-like Domain
- TM Region
- TSR
- Cys-rich Domain
- EGF Domain
Previous research

- mRNA expression of ADAMTS 1, 4 and 5 in human CNS
- ADAMTS-1 is over-expressed in the CNS in Down's syndrome and Alzheimer's disease
- mRNA expression of ADAMTS 1, 4 and can be regulated by cytokines in a range of cell lines
- Beta amyloid induces ADAMTS4 expression by astrocytes
- ADAMTS 1 exhibits anti-angiogenic activity
Plan of investigation

- Use an animal model of stroke to investigate the expression of ADAMTS enzymes
- Assess whether these enzymes might act as potential new therapeutic targets
MCAo model

- Adult male Sprague Dawley rats
- Intraluminal thread inserted into middle cerebral artery under anaesthesia.
- Thread withdrawn after 90 minutes to allow reperfusion
- Sham operated animals used as controls
Expression of proteases and cytokines in an animal model of stroke

- Rats are anaesthetised and the middle cerebral artery is occluded for 90 minutes
- Blood flow is restored and the brains are taken at 6, 24 and 120 hours
- 4 animals in each group including a sham operated group
- Each brain is dissected into two hemispheres and each is halved
- Samples are coded and processed anonymously
- Samples are assessed for mRNA using real time PCR and sections are also taken for immunostaining to look at protein in the tissue
ADAMTS-1 expression following MCAo

Relative expression (2^-dCT)

- sham
- CL (6 hours)
- IL (6 hours)
- sham (24 hours)
- CL (24 hours)
- IL (24 hours)
- sham (5 days)
- CL (5 days)
- IL (5 days)

* * *
ADAMTS-4 expression following MCAo

![Graph showing relative expression (2^-dCT) for sham, CL, and IL groups at 6 hours, 24 hours, and 5 days post-MCAo. Significant differences are indicated by * and **.]
ADAMTS-5 expression following MCAo

Relative expression ($2^{\Delta_{dCT}}$)

- sham
- CL
- IL

6 hours 24 hours 5 days

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Cytokine expression, 24 hours post MCAo in the rat model

IL-1β

![Graph showing IL-1β expression levels for sham-operated and MCAo groups.](image)

TNF

![Graph showing TNF expression levels for sham-operated and MCAo groups.](image)
Immunostaining of stroke brain

ADAM 17

Astrocytes
Astrocytes in the stroke tissue
ADAMTS-1 expression by TNFα treated human astrocytes
Immunocytochemistry on primary human astrocytes

- GFAP
- ADAMTS-1
- ADAMTS-4
- ADAMTS-5
Summary

- ADAMTS-1 and -4 are significantly increased following MCAo
- mRNA expression is increased as early as 6 hours post MCAo, although highest expression is at 24 hours
- Astrocytes express ADAMTS-1, 4 and -5
- Expression of ADAMTS-1 and -4 is increased by TNFα
- Further work will investigate whether inhibitors of these enzymes decrease the tissue damage and improve the outcome from stroke
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