Perlecan domain V is neuroprotective and proangiogenic following ischemic stroke in rodents

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Stroke

A stroke is the rapid loss of brain function due to disturbance in the blood supply to the brain.

A stroke is a medical emergency and can cause permanent neurological damage, complications, and death.
Ischemic stroke

- A condition resulting from occlusion of brain vasculature
  
- Manifests as an ischemic core of rapid cell death, surrounded by a vulnerable penumbral region
  
- Angiogenic blood vessels serve as a physical scaffold for neurons to migrate toward the ischemic core
  
- Therapies such as pharmaceuticals, stem cells, and growth factors have attempted to capitalize on neurovascular repair concepts to promote stroke recovery

Pharmaceutical and growth factor therapies raise questions of potentially serious systemic side effects, drug interactions, and contraindications

Lo EM, et al 2003
Lo EM, et al 2008
Ohab JJ, et al 2006
Many factors that prevent cell death also inhibit repair, or vice versa, depending upon when they are administered after stroke.

**NMDA receptor antagonists and protease inhibitors**
- Neuroprotective
- Detrimental to repair  
  
  Zhang ZG, et al, 2000

**Vascular endothelial growth factor (VEGF)**
- Neuroprotective
- Enhances angiogenesis
- Neurogenesis
- Disrupts blood-brain barrier stability
- Promotes brain edema
- Brain infarct size  
  
Perlecan domain V (DV)

- Perlecan
  - Vascular extracellular matrix
  - Maintain vascular homeostasis
  - Promote growth factor
    - Endothelial growth
    - Re-generation

- Perlecan also contains the antiangiogenic C-terminal protein fragment domain V (DV)

DV as know as Endorepellin

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<tr>
<th>HUVEC control</th>
<th>+ Endorepellin</th>
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- Perlecan is required for brain angiogenesis and neurogenesis in brain atrophy

DV has not been studied angiogenesis and neurogenesis in the stroke brain???
Hypothesis

To identify where perlecan domain V is neuroprotective and proangiogenic following ischemic stroke.
Aim 1. To test whether the perlecan domain V is required for ischemia stroke

Aim 2. To identify whether the perlecan domain V is mediated neuroprotection and angiogenesis by VEGF and VEGFR

Aim 3. To identify whether the perlecan domain V effects are mediated angiogenesis via the α5β1 integrin
Acute stroke model

RAT

Middle Cerebral Artery Occlusion

Vasoconstrictor: endothelin-1
Perlecan DV is upregulated after stroke in rodents
Perlecan DV is upregulated after stroke in rodents

Mice: Perlecan hypomorphic mutations (pln −/−)

TTC stain

**Mean ischemic lesion volume (mm²)**
Administered DV reaches stroke brain tissue

Human DV inject by I.P

![Image of human DV inject by I.P](image)

**A**

Stroke peri-infarct DV treated

Stroke tissue DV treated

**B**

vWF

DV (anti-HIS)

Merge

**C**

vWF + DV (anti-HIS) + DAPI

**D**

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<tr>
<th></th>
<th>PBS</th>
<th>DV</th>
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<tr>
<td>Anti-DV</td>
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<tr>
<td>Anti-HIS</td>
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<td>Anti-GAPDH</td>
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85 kDa

36 kDa

**E**

IR-800 dye control

IR-800 DV

![Image of IR-800 dye control and IR-800 DV](image)
DV is neuroprotective
DV is neuroprotective
DV restores post-stroke motor function
Aim 1.

To test whether the perlecan domain V is required for ischemia stroke

- Perlecan DV is upregulated after stroke in rodents
- Administered DV reaches stroke brain tissue
- DV is neuroprotective
- DV restores post-stroke motor function
Aim 2.
To identify whether the perlecan domain V is mediated neuroprotection and angiogenesis by VEGF and VEGFR
DV neuroprotection is VEGF and VEGFR mediated

- DV activity outside of the brain has been linked to the suppression of VEGF signaling
- VEGF is known to be neuroprotective

**VEGFR blocker**: PTK787/ZK 222584
DV does not increase post-stroke blood-brain barrier permeability
DV is neuroprotective in vitro via enhancement of VEGF secretion from brain endothelial cells
DV increases brain angiogenesis in a VEGF- and VEGFR-dependent fashion
DV increases brain angiogenesis in a VEGF- and VEGFR-dependent fashion
Aim 2.
To identify whether the perlecan domain V is mediated neuroprotection and angiogenesis by VEGF and VEGFR

- DV neuroprotection is VEGF and VEGFR mediated
- DV does not increase post-stroke blood-brain barrier permeability
- DV increases brain angiogenesis in a VEGF- and VEGFR-dependent fashion
Aim 3.
To identify whether the perlecan domain V effects are mediated angiogenesis via the α5β1 integrin
DV effects are mediated via the α5β1 integrin in vivo.

Perlecan, DV’s parent molecule, increases α5β1 integrin expression in brain endothelial cell
Perlecan supports β1 integrin–mediated cell adhesion via its DV region

DV effects are mediated via the α5β1 integrin in vivo.
DV’s effects are mediated via the α5β1 integrin in vitro

α5β1-specific binding peptide CRRETAWAC

optical biosensor
Conclusion
Discussion

Why not to use BrdU?
Discussion

DV and VEGF ???