Promoting neurovascular repair after ischemic stroke by targeting pericytes

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BACKGROUND

Ischemic stroke constitute a major cause of death and disability of the adults worldwide. Unfortunately, there is no efficient therapy so far. Ischemic stroke triggers endogenous neurovascular restorative responses within the ischemic tissue as an attempt from the brain to recover. Angiogenesis is one of such compensatory mechanisms, which involves close interactions between brain endothelial cells and pericytes. Pericytes play major roles in regulating the cerebral blood flow, angiogenesis, microvasculature stability, and blood-brain barrier. After Ischemic stroke, pericytes detaches from brain endothelial cells, leading to neurovascular impairment. Therefore, strategies aiming to promote vascular stability by improving pericytic density on the microvasculature have been proposed to constitute a promising therapeutic approach.

OBJECTIVES

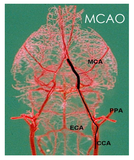
Evaluating the therapeutic potential of Vascular Endothelial Growth factor VEGF isoform-B (VEGF-B) in ischemic stroke and deciphering the underlying mechanims with an empahsis on the pericytes.

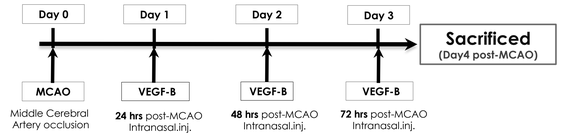
OBJECTIVES:

- To investigate the effects of VEGF-B on vascular stability and tissue damage *in vivo*.
- To dissect the signaling pathway of VEGF-B / VEGFR-1 in pericytes *in vivo* and *in vitro*.

MATERIALS & METHODS

- **Ischemic stroke:** Adult C57BLj6 mice were subjected to transient middle cerebral artery MCA occlusion (MCAo).
- **Treatment:** Mice were treated with VEGF-B (4 µg/day beginning 24 hours after MCAo onset for 3 successive days; intranasal) and control mice received saline solution.
- **Cells** - To decipher the underlying mechanisms primary human brain micorvascular pericyte (HBPV) cells were used.





RESULTS

1. VEGF-B reduces neurovascular injury after ischemic stroke

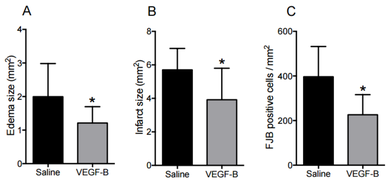


Figure 1: (A, B) Cresyl violet staining was used to evaluate the edema size and infarct volume demonstrating that VEGF-B significantly decreases the edema and infarct sizes. **(C)** Quantitative analysis of Fluoro-Jade B positive cells in the ischemic region shows that there is a significant decrease in the number of degenerating neurons in VEGF-B treated animals.

2. VEGF-B enhances brain microvasculature density and their coverage by pericytes

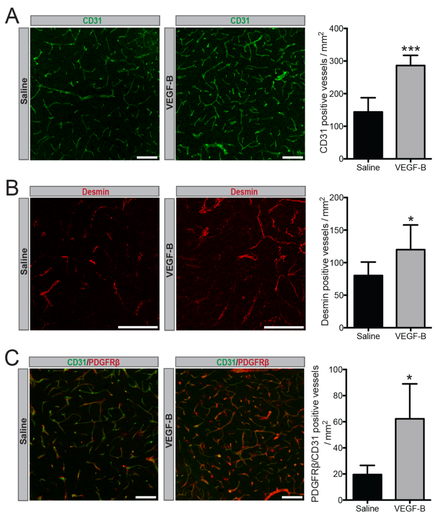


Figure 2. (A,B,C) Immunofluorescence representative images showing CD31 (endothelial cell marker; green), desmin (pericyte marker; red), and PDGFRβ (pericyte marker; red). shows: **(A)** Stereological quantification shows increased brain microvasculature density in the ipsilateral hemisphere after VEGF-B treatment. **(B)** Stereological quantification shows enhanced absolute abundance of pericytes in the ipsilateral hemisphere after VEGF-B treatment. **(C)** co-localisation studies show an increase in the number of brain microvasculature (CD31+ staining; green) covered by pericytes (PDGFRβ+ staining; red) in the ipsilateral hemisphere after VEGF-B treatment.

3. VEGF-B attenuates structural damage of the microvasculature

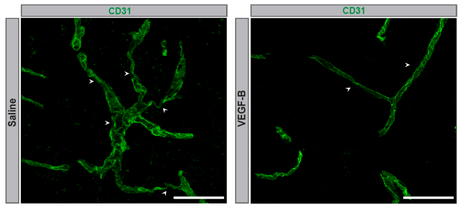


Figure 3. Laser-scan confocal anlaysis examining the morphology of the brain microvasculature (CD31+ staining; green) in the ipsilateral hemisphere shows that majority of the brain microvasculature in the saline-treated animals displays several constrictive bulb-like structures (white arrows), while in the VEGF-B-treated animals such structures were absent.

4. Association of brain endothelial cells and pericytes increases following VEGF-B administration

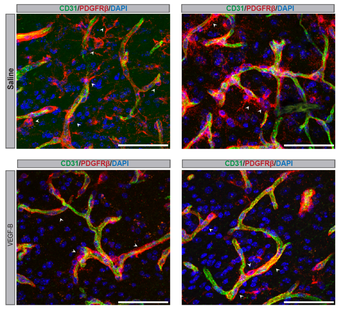


Figure 4. Laser-scan confocal analysis of triple immunofluorescence staining for CD31 (endothelial cell marker; green)/PDGFRβ (pericyte marker; red)/DAPI (nucleus marker; blue) shows that in the ipsilateral hemisphere of saline-treated animals pericytes are activated presenting a ramified-like morphology and are detaching from endothelial cells by protruding towards the parenchyma (white arrows). VEGF-B reduces the activation of pericytes that are now more firmly attached to endothelial cells, and enhances the ensheathment of brain microvasculature by pericytes **(white arrows)**.

5. VEGF-B rescues pericyte survival and metabolism upon OGD

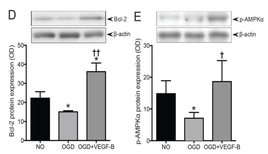


Figure 5. (D) Western blot analysis shows that VEGF-B restores the expression of Bcl-2 in HBMVP exposed to the OGD. **(E)** Western blot analysis shows that VEGF-B significantly enhances the phosphorylation (i.e activation) of AMPKα in HBMVP exposed to the OGD conditions.

6. VEGFR-1 is predominantly expressed in brain pericytes and its expression is regulated by hypoxia

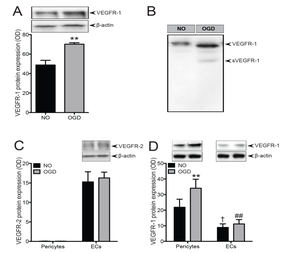


Figure 6. Western blot analysis shows that: **(A)** VEGFR-1 expression in HBMVP is specifically induced. **(B)** OGD induces expression of the soluble form of VEGFR-1 (sVEGFR-1). **(C)** HBMVP do not express VEGFR-2, whereas VEGFR-2 is highly expressed in brain endothelial cells and its expression level remains unchanged upon OGD. **(D)** VEGFR-1 expression level is significantly higher in HBMVP compared to endothelial cells.

7. VEGF-B attenuates pericyte cell loss after OGD

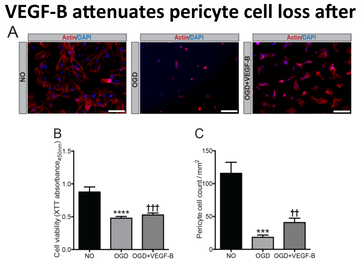


Figure 7. (A) Representative images of F-actin staining show normal actin cytoskeleton morphology in normoxia conditions, disrupted morphology in OGD, and restored morphology in OGD following VEGF-B stimulation. **(B)** XTT cell viability assay shows that VEGF-B significantly enhances the survival of HBMVP exposed to OGD. **(C)** Absolute cell count analysis shows that VEGF-B potently rescues cell loss induced by OGD.

8. VEGF-B promotes restorative angiogenesis

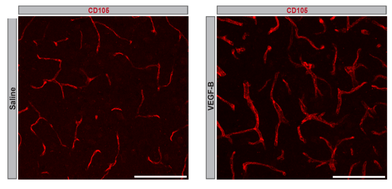


Figure 8. Representative images of CD105 (proliferating endothelial cell marker) immunofluorescence staining outline an increase in the number of CD105+ brain microvasculature in the ischemic stroke region of mice treated with VEGF-B.

9. VEGF-B enhances the capacity of pericytes exposed to OGD to produce molecules involved in promoting reparative angiogenesis

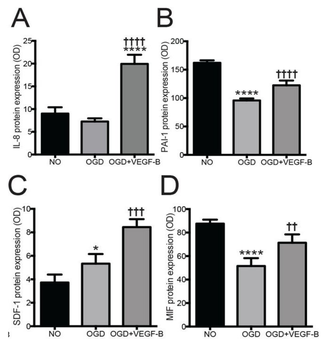


Figure 9. Cytokine/chemokine assay demonstrates that: **(A)** VEGF-B induced protein expression of IL-8 in HBMVP (brain pericytes) exposed to the OGD conditions. **(B)** OGD reduces expression of PAI-1 in HBMVP exposed to the OGD conditions. **C)** OGD increases expression of SDF-1 in HBMVP exposed to the OGD conditions. **D)** OGD reduces expression of MIF in HBMVP exposed to the OGD conditions.

CONCLUSION

- VEGF-B, promoted the formation of stable microvasculature within the ischemic tissue by specifically enhancing the survival of pericytes and their interaction with brain endothelial cells.
- It induces the expression of soluble factors involved in promoting reparative angiogenesis.
- The effects of VEGF-B are mediated via its specific receptor VEGFR-1 that is predominately expressed in brain pericytes.
- Our study unraveled an unknown role of VEGF-B/VEGFR-1 signalling in regulating the function of pericytes.

SUMMARY: Our findings suggest that strategies aiming to stimulate the endothelial-pericyte crosstalk constitute a promising therapeutic approach to promote neurovascular repair upon ischemic stroke.

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