VEGF enhance the permeability of the blood-brain barrier

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Objective: The blood-brain barrier (BBB) is a tightly regulated barrier that strictly controls the exchanges between the blood and brain components. The vascular endothelial growth factor (VEGF) is one of the most important growth factors in the process of angiogenesis and vasculogenesis. We aim to (1) increase the permeability of BBB by the injection of VEGF so as to make the central nervous system (CNS) drug delivery convenient; (2) find an effective method for the measurement of the extent of the BBB permeability by MRI in vivo.

Methods and materials: In this study, we used 18 KM mice, which were divided into two groups: 12 in histopathology group and 6 in MRI group, respectively. The 12 mice (18-22g) in the histopathology group were randomized into 3 subgroups. Group 1 received 200ul saline only, group 2 & group 3 received recombinant human VEGF-165 (0.015mg/ml, dissolved in saline) 200ul through the tail vein and was treated for 4h and 12h respectively. The mice received 0.075ml Evans Blue (2%; 3ml/kg) after treated as above and allowed Evans Blue to circulate for 30 minutes. The 12 mice in histopathology group were sacrificed 0.5h after the injection of Evans Blue and perfused with saline and paraformaldehyde (PFA) sequentially. Subsequently, the brains were removed and placed in PFA on ice. After 12 hours infusion in PFA, brains were washed with PBS (0.01M) three times every 10 minutes. Then the brains were coated with agarose (4%), and vibratio microtome (Leica, Germany) was used to get the brain slides. The slides were immediately put under the fluorescence microscope thereafter to detect red fluorescence. The 6 mice in MRI group were randomized into 2 groups (Group A and Group B), 3 in each group. Group A was pretreated with saline and Group B with VEGF. MRI (Bruker BioSpec 7T/30cm system, Bruker, Ettlingen, Germany) was performed 4 hours and 12 hours later respectively for these two groups. Isoflurane (1.4%) was administered during the MRI examination procedure. The 6 mice were examined by MRI before and 5min, 10min, 15min after the injection of Gd-DTPA, respectively. Paravision 5.0 software and volume coil (outer diameter 44mm and inner diameter 23mm) were used. MRI sequences including RARE-T2 (TR3000, TE45, slice thickness 1mm, FOV 18mm, Matrix 256*256), MSME-T1 (TR 300, TE 11, slice thickness 1mm, FOV 18mm, Matrix 256*256) and DWI (TR 3000, TE 30, slice thickness 1mm, FOV 25mm, Matrix 128*128, B value 100, 200, 400, 600, 800, and 1000s/mm^2, No. of diffusion direction 1).

Results: (1) Histological results showed Group 3 had a significant higher permeability of blood vessel than Group 1 (Figure 1). Group 2 had a slightly higher permeability of blood vessel than Group 1 (Figure 1), the difference was not obvious. (2) MRI examination showed Group B had a higher signal intensity of brain parenchyma ($P<0.001$) than Group A (Figure 2). The extravasation of Gd-DTPA observed within the brain showed an impairment of the BBB integrity. Minimal edema between these two groups in MRI group was also investigated, however, no significant difference was found from picture obtained.

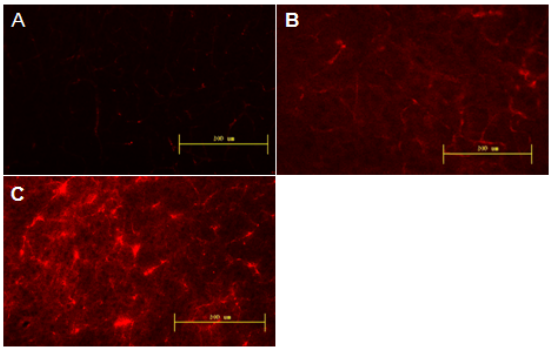

Figure 1. *Permeability of blood vessel in histopathology group. 30 minutes after intravenous injection of Evans blue dye (2%, 3ml/kg), the red fluorescence was not detected elsewhere besides blood wall in the CNS of group 1 (A). By contrast, group 2 has a slightly higher fluorescence intensity (B). After 12h pretreatment with VEGF, red fluorescence is diffused around vessels in group 3 (C). Note that, all slides are taken approximately at the same portion of the brain and with the same exposure time.*

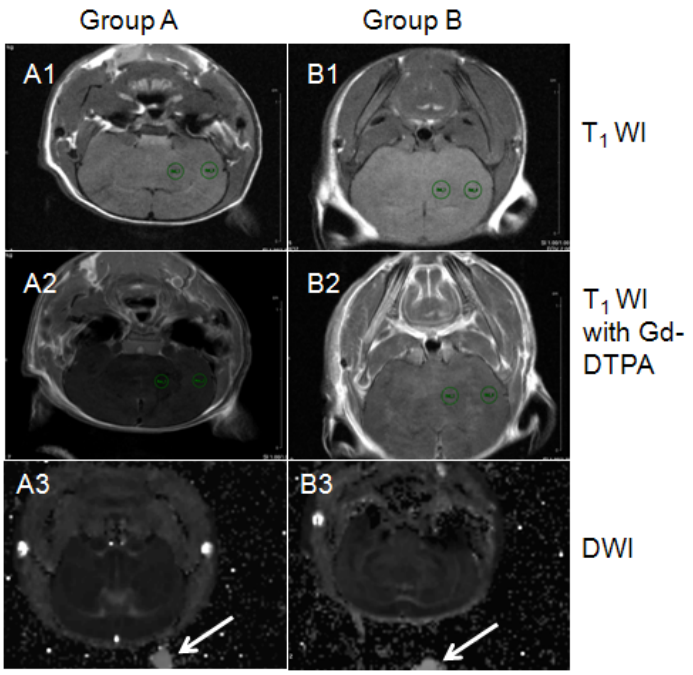

Figure 2. *Permeability of blood vessels by MRI examination. MRI scans generated before administration of contrast agent (Gd-DTPA) in two groups of mice (A_1 and B_1) and the others scanned immediately after the injection of Gd-DTPA (A2 and B2). Group A was pretreated with 200ul saline. Group B was pretreated with 200ul VEGF (0.015mg/ml) for 12hours. ROI (region of interest) is for the measurement of signal intensity. ROI1 stands for the region of basal ganglia and ROI2 stands for the region of cerebral cortex. DWIs (A3 and B3) of these two groups show no significant difference. Arrowheads indicate water tube.*

Conclusions: (1) The exogenous VEGF can enhance the BBB permeability and may induce neovascularization. The permeability of the BBB can be modulated by venous injection of VEGF and may be a new direction to deliver drugs to the CNS. (2) MRI has a good sensitivity in the detection of BBB leakage compared with histological method and may be an ideal method for the measurement of the permeability of the BBB in vivo.

Reference:

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