Title: **Dl-3-N-butylphthalide promotes angiogenesis in an optimized model of transient ischemic attack in C57BL/6 mice**

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Abstract:

Transient ischemic attack (TIA) has been widely regarded as a clinical entity. Even though magnetic resonance imaging (MRI) results of TIA patients are negative, potential neurovascular damage might be present, and may account for long-term cognitive impairment. Animal models that simulate human diseases are essential tools for in-depth study of TIA. This study aims to develop an optimized TIA model in C57BL/6 mice to explore the microscopic evidence of ischemic injury after TIA, and investigate the therapeutic effects of Dl-3-N-butylphthalide (NBP) on TIA. C57BL/6 mice were subjected to varying durations of ischemia (7, 8, 9 or 10 min) using the intraluminal middle cerebral artery occlusion (MCAO) method. Cerebral artery occlusion and reperfusion were assessed by laser speckle contrast imaging. TIA and ischemic stroke were distinguished by neurological testing and MRI examination at 24 h post-operation. Neuronal apoptosis was examined by TUNEL staining. Images of submicron vascular networks were obtained via micro-optical sectioning tomography. Subsequently, the mice were randomly assigned to a sham-operated group, a vehicle-treated TIA group or an NBP-treated TIA group. Vascular density was determined by immunofluorescent staining and fluorescein isothiocyanate method, and the expression of angiogenic growth factors were detected by western blot analysis. We found that an 8-min or shorter period of ischemia induced neither permanent neurological deficits nor MRI detectable brain lesions in C57BL/6 mice, but histologically caused neuronal apoptosis and cerebral vasculature abnormalities. NBP treatment increased the number of CD31+ microvessels and perfused microvessels after TIA compared with the vehicle-treated group. NBP also up-regulated the expression of VEGF, Ang-1 and Ang-2. In conclusion, 8 min or shorter cerebral ischemia induced by the suture MCAO method is an appropriate TIA model in C57BL/6 mice, which conforms to the definition of human TIA, but causes microscopic neurovascular impairment. NBP treatment increased the expression of angiogenic growth factors, promoted angiogenesis and improved cerebral microvessels after TIA. Our study provides new insights on the pathogenesis and potential treatments of TIA.