Using Functional and Molecular MRI Techniques to Detect Neuroprotection by Pinocembrin in Rats Subjected to Traumatic Brain Injury

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Target audience: Researchers and clinicians who are interested in functional and molecular imaging of TBI.

Introduction

Traumatic brain injury (TBI) results in primary injury and secondary injury cascades, such as ischemia, progressive neurodegeneration, persistent inflammation, glial hypertrophy and proliferation, and cerebrovascular dysfunctions[1]. Currently, no drug is effective for treatment of TBI. Flavonoid pinocembrin was shown to be neuroprotective after cerebral ischemia in rats[2]. However, its potential protection in TBI has not been tested. In this study, we subjected rats to a controlled cortical impact (CCI)-TBI model and used several noninvasive structural, functional, and molecular MRI techniques to examine whether pinocembrin can provide neuroprotection.

Methods

Thirty-one adult male SD rats were randomly divided into four groups: sham group (n=5), vehicle-treated TBI group (n=6), TBI group treated with 5 mg/kg pinocembrin (n=10), and TBI group treated with 10 mg/kg pinocembrin (n=10). Rats from the vehicle and pinocembrin groups were subjected to craniotomy plus CCI surgery (3-mm impact tip, velocity of 5 m/sec, deformation depth of 5 mm, and impact duration of 65 msec) under isoflurane anesthesia. Rats from the sham group received scalp incision, but the skull was kept intact. Pinocembrin was injected through the tail vein at 30 min and 1, 2, and 3 days after CCI. MRI data were acquired on a 4.7T animal imager, using T2-weighted (T2w), T2*-weighted (T2*w), T2, isotropic apparent diffusion coefficient (ADC), CBF, and amide proton transfer (APT)-weighted (APTw) sequences. APT imaging is a novel molecular MRI method that can noninvasively detect endogenous mobile protein concentration and tissue pH changes[3]. MRI was performed at 1 h and 1, 3, 7, 14, and 28 days after TBI. Lesion volume was measured, using areas of T2w MRI hyperintensity, at various time points. Immunofluorescence was used to detect microglia/macrophage and astrocyte activation.

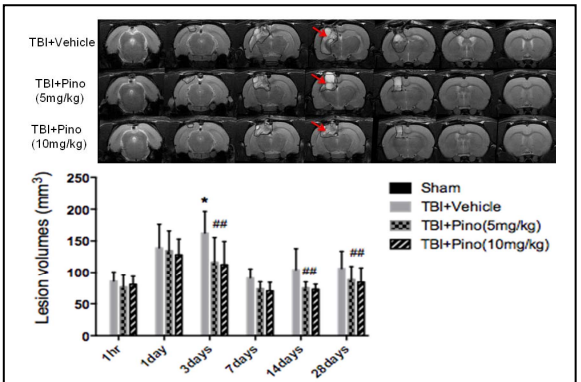

Fig. 1. (Top) T2-weighted images at 28 days after TBI. (Bottom) Lesion volume as a function of days after TBI.

Results and Discussion

At 3, 14, and 21 days after TBI (**Fig. 1**), the lesion volumes were significantly smaller in the pinocembrin groups than in the vehicle group, but there was no significant difference between the 5 mg/kg and 10 mg/kg pinocembrin-treated groups. Pinocembrin improved performance of all three behavioral tests on day 3 after TBI. Only mNSS and rotarod performance were improved on day 28.

At 3 days after TBI, the TBI lesion became heterogeneous (**Fig. 2A**), with areas of high and low APTw signal intensities. Notably, the APTw signal intensity of the perilesional region increased dramatically in the vehicle-treated TBI group, but decreased in the pinocembrin-treated TBI group (**Fig. 2B**). At 7 days after TBI, the ADC, which probes cellularity, had a significantly lower signal in the pinocembrin-treated TBI group than in the vehicle-treated TBI group (**Fig. 2C**).

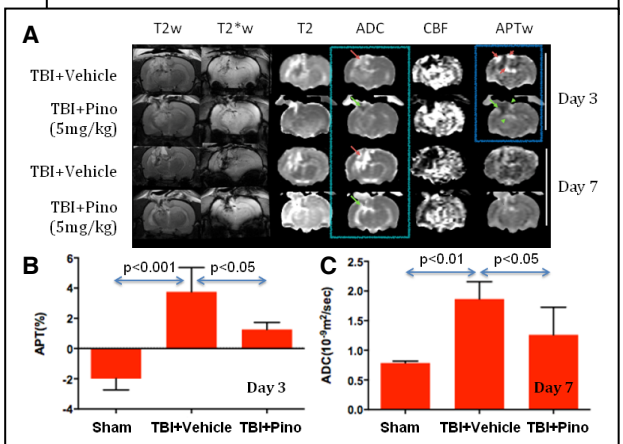

Fig. 2. (A) Multi-parametric MRI results for a vehicle-treated TBI rat and a pinocembrin-treated TBI rat at 3 and 7 days after TBI. (B) APTw signals for three groups at 3 days after TBI. (C) ADC signals for three groups at 7 days after TBI.

Consistent with the changes of APTw signal in the perilesional region, fluorescence intensities of Iba1 and GFAP were significantly higher in the vehicle-treated TBI group, but significantly decreased due to the pinocembrin treatment at 3 days after TBI (**Fig. 3**). These histopathological results suggested that increased APTw signal could be attributed to the increased secondary inflammatory response, evidenced by microglial and astrocyte activation.

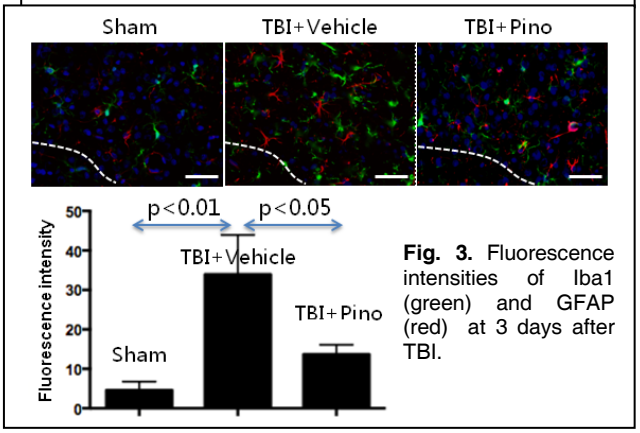

Fig. 3. Fluorescence intensities of Iba1 (green) and GFAP (red) at 3 days after TBI.

Conclusion

Flavonoid pinocembrin is neuroprotective in the CCI-TBI model in rats. The APT-MRI signal could have potential clinical applications as a unique, sensitive biomarker for identification and assessment of neuroinflammation in the TBI model.

References: (1) Immonen et al. JCBFM 30 (2010)1318. (2) Shi et al. Life Sci. 88 (2011)521. (3) Zhou et al. Nat. Med. 9 (2003)1085.