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Farnesoid X receptor knockout protects brain against ischemic injury through reducing neuronal apoptosis in mice

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Abstract

Background: Farnesoid X receptor (FXR) is a nuclear receptor that plays a critical role in controlling cell apoptosis in diverse diseases. Previous studies have shown that knocking out *FXR* improved cardiac function by reducing cardiomyocyte apoptosis in myocardial ischemic mice. However, the role of FXR after cerebral ischemia remains unknown. In this study, we explored the effects and mechanisms of *FXR* knockout (KO) on the functional recovery of mice post cerebral ischemia-reperfusion.

Methods: Adult male C57BL/6 wild type and *FXR* KO mice were subjected to 90-min transient middle cerebral artery occlusion (tMCAO). The mice were divided into five groups: sham, wild-type tMCAO, *FXR* KO tMCAO, wild-type tMCAO treated with calcium agonist Bayk8644, and *FXR* KO tMCAO treated with Bayk8644. FXR expression was examined using immunohistochemistry and Western blot. Brain infarct and brain atrophy volume were examined at 3 and 14 days after stroke respectively. Neurobehavioral tests were conducted up to 14 days after stroke. The protein levels of apoptotic factors (Bcl-2, Bax, and Cleaved caspase-3) and mRNA levels of pro-inflammatory factors (TNF-α, IL-6, IL-1β, IL-17, and IL-18) were examined using Western blot and RT-PCR. TUNEL staining and calcium imaging were obtained using confocal and two-photon microscopy.

Results: The expression of FXR was upregulated after ischemic stroke, which is located in the nucleus of the neurons. *FXR* KO was found to reduce infarct volume and promote neurobehavioral recovery following tMCAO compared to the vehicle. The expression of apoptotic and pro-inflammatory factors decreased in *FXR* KO mice compared to the control. The number of $NeuN^+/TUNEL^+$ cells declined in the peri-infarct area of *FXR* KO mice compared to the vehicle. We further demonstrated that inhibition of FXR reduced calcium overload and addition of ionomycin could reverse this neuroprotective effect in vitro. What is more, in vivo results showed that enhancement of intracellular calcium concentrations could aggravate ischemic injury and reverse the neuroprotective effect of *FXR* KO in mice.

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