STAT3 Phosphorylation Mediated SOD2 Up-Regulation by Electroacupuncture Attenuates Ischemic Oxidative Damage via Cannabinoid CB1 in Stroke Mice

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Background: Oxidative stress induced by mitochondria dysfunction plays a key role in the pathogenesis during ischemic/reperfusion injury. SOD2, as known as manganese superoxide dismutase, is an important antioxidant enzyme to attenuate the mitochondrial oxidative stress. The SOD2 activation could reduce experimental ischemic injury significantly. However, SOD2 activation involved the cerebral ischemic tolerance is unknown. Our previous studies have demonstrated that electroacupuncture (EA) pretreatment elicits the neuroprotective effect against cerebral ischemic injury through cannabinoid receptor type 1 receptor (CB1R) and its mediated protective signaling pathways such as pro-survival pathway the signal transducer and activator of transcription 3 (STAT3). In the present study, we tried to investigate whether manganese superoxide dismutase were involved in the EA pretreatment via CB1R activation in stroke mice and whether CB1R mediated SOD2 activation via STAT3 phosphorylation.

Methods: At 2 h after EA pretreatment, focal cerebral ischemic injury was induced by transient middle cerebral artery occlusion for 60 min in C57BL/6 mice. The expression of SOD2 in the penumbra was assessed by western-blot and immuneflourescent staining at 2 h after reperfusion. In the presence or absence of SOD2-siRNA, the neurological deficit score, the infarct volume, the TUNEL staining and oxidative stress (the content of ROS, MDA, 8-OHdG, nitrotyrosine) were evaluated. Furthermore, the SOD2 protein expression and phosphorylation of STAT3 at 705Y were also determined in the presence or absence of two CB1R antagonists AM251 and SR141716A, as well as two CB1R agonists ACEA and WIN55212-2.

Results: EA pretreatment up-regulated the SOD2 protein expression （+84.4%, $P<0.05$） and increased SOD2 positive neuronal cells at 2 h after reperfusion. EA pretreatment attenuated the generation of oxidative injury products (MDA, -33.9%, $P<0.05$;8-OHdG, -37.7%, $P<0.05$;ROS, -28.9%, $P<0.05$; nitrotyrosine, -22.0%, $P<0.05$) and reduced the DHE oxidation positive cells. EA pretreatment also inhibited the cellular apoptosis (-34.5%, $P<0.05$), and induced a neuroprotective effect against ischemic damage (infarct size, -32.3%, $P<0.05$;neurological deficit, -36%, $P<0.05$). However these beneficial effects of EA pretreatment were reversed by knockdown of SOD2 ($P>0.05$). The administration of two CB1R antagonists blocked the up-regulation of SOD2 induced by EA pretreatment (AM251, -22.4%,

P<0.05;SR141716, -37.3%, *P*<0.05 respectively) and two CB1R agonists increased SOD2 protein expression (WIN55-212-2, +33.1%, *P*<0.05;ACEA, +56.1%, *P*<0.05 respectively). Moreover, EA pretreatment increased STAT3 phosphorylation level at Y705 (+88.0%, *P*<0.05). Two CB1R antagonists also reversed the increased STAT3 phosphorylation level at Y705 (AM251, -26.2%, *P*<0.05;SR141716, -38.6%, *P*<0.05 respectively) while two CB1R agonists increased this level (WIN55-212-2, +94.7%, *P*<0.05;ACEA, +14.6%, *P*<0.05 respectively).

Conclusions: Cannabinoid CB1 receptor mediated SOD2 up-regulation by EA pretreatment attenuates ischemic oxidative damage via STAT3 phosphorylation in stroke mice, which may represent one new mechanism of EA pretreatment-induced neuroprotection against cerebral ischemia in mice.

Key Words: Electroacupuncture; Cerebral ischemia; SOD2; oxidative damage; Cannabinoid CB1 receptor