Epigenetic drugs targeting acetylation of NF-KappaB and histones: a promising strategy to reduce post ischemic brain injury

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Nuclear factor-kappaB (NF-κB) p50/RelA is a key molecule with dual effect in the progression of ischemic stroke. In harmful ischemia, but not in preconditioning insult, neurotoxic activation of p50/RelA is characterized by RelA-specific acetylation at Lys310 (K310) and deacetylation at other Lys residues. The derangement of RelA acetylation is associated with reduced histone acetylation and NF-κB-dependent activation of the pro-apoptotic *Bim* promoter. With the aim to produce neuroprotection by correcting altered acetylation of RelA and histone proteins in brain ischemia, we studied the combination of the clinically used histone deacetylase (HDAC) inhibitors, entinostat (MS-275) or valproic acid (VPA), in combination with the sirtuin-1 activator resveratrol. We used the mice model of transient middle cerebral artery occlusion (MCAO) and cultured neurons exposed to oxygen glucose deprivation (OGD). The combined use of a HDAC inhibitor, MS-275 or VPA, and resveratrol elicited a synergistic neuroprotection in primary neurons exposed to OGD. Particularly, in cells treated with MS-275 and resveratrol this beneficial effect correlated with the MS-275 capability to increase total RelA acetylation and the resveratrol capability to reduce RelA K310 acetylation through an AMP-activated protein kinase/sirtuin 1 pathway. Thus, reproducing the acetylation state of RelA peculiar of preconditioning ischemia. Moreover, neurons coexposed to MS-275 and resveratrol also recovered the optimal histone H3 acetylation.

Neuroprotection was also reproduced in mice subjected to MCAO and treated with MS-275 (20 μg/kg and 200 μg/kg), resveratrol (6800 μg/kg) or VPA (20 mg/kg), individually. However, when combining MS-275 or VPA and resveratrol at 100-fold lower concentrations a significant reduction in both infarct volume and neurological deficits was observed. Moreover, the combined treatment of MS-275 and resveratrol was effective even when administered 7 hours after the stroke onset.

Additionally, chromatin immunoprecipitation analysis in cerebral cortices harvested from mice exposed to MCAO and treated with MS-275 and resveratrol or vehicle showed that the treatment induced the shifiting of RelA binding from the *Bim* to the *Bcl-x_L* promoter.

Epigenetic therapy targeting RelA and histone acetylation may be a promising strategy to limit post ischemic injury with an extended therapeutic window.