# Targeted acetylation of NF-kappaB and histones by epigenetic drugs reduces post-ischemic brain injury in mice with an extended therapeutic window

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Nuclear factor-kappaB (NF-κB) p50/RelA is a key molecule with a 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. We found that the derangement of RelA acetylation induced NF-κB binding to the pro-apoptotic Bim promoter and its detachment from the anti-apoptotic Bcl-xL promoter.
With the aim of producing neuroprotection by correcting altered acetylation of RelA in brain ischemia, we combined the pharmacological inhibition of histone deacetylase (HDAC) 1–3, the enzymes known to reduce global RelA acetylation, and the activation of sirtuin 1, endowed with a specific deacetylase activity on the K310 residue of RelA.
To afford this aim, we tested the clinically used HDAC 1–3 inhibitor entinostat (MS-275) and the sirtuin 1 activator resveratrol. In neurons exposed to oxygen glucose deprivation (OGD), the combined use of MS-275 (0.1 μM) and resveratrol (3 μM) elicited a synergistic neuroprotection that correlated with the capability of MS-275 to increase the total RelA acetylation and the capability of resveratrol to activate AMP-activated protein kinase and sirtuin 1, resulting in the deacetylation of RelA K310. The synergistic treatment reproduced the acetylation state of RelA peculiar of preconditioning ischemia. Neurons coexposed to these drugs also totally recovered the optimal histone H3 acetylation. Neuroprotection was reproduced in mice subjected to middle cerebral artery occlusion (MCAO) and treated with MS-275 (20 μg/kg and 200 μg/kg) or resveratrol (6800 μg/kg) individually. However, the administration of lower doses of MS-275 (2 μg/kg) and resveratrol (68 μg/kg) synergistically reduced infarct volume and neurological deficits. Importantly, the treatment was effective even when administered 7 h after the stroke onset. Chromatin immunoprecipitation analysis of cortices harvested from treated mice showed that the RelA binding shifted from the Bim to the Bcl-xL promoter. Our study reveals that epigenetic therapy shaping acetylation of both RelA and histones may be a promising strategy to limit post-ischemic injury with an extended therapeutic window.