Synergistic effect of the epigenetic drugs resveratrol and Valproic acid in reducing post ischemic brain injury.

L. Faggi[1], G. Pignataro[2], A. Lanzillotta[1], E. Parrella[1], V. Porrini[1], A. Vinciguerra[2], P. Cepparulo[2], M. Benarese[1], M. Mota[1], L. Annunziato[2], PF. Spano[1,3], M. Pizzi[1,3].

1 Dept. of Molecular and Translational Medicine, University of Brescia, Italy
2 Dept. of Neuroscience, Federico II University of Naples, Italy
3 IRCCS, S. Camillo Hospital, Venice, Italy.

Background:
Nuclear factor-kappaB (NF-κB) p50/RelA plays a dual role in the progression of ischemic stroke. It is activated both in protective and lethal events, with a different acetylation profile. In harmful ischemia, neurotoxic activation of p50/RelA is characterized by general deacetylation of RelA, but site-specific acetylation at Lys310 (K310). This peculiar RelA acetylation is associated with a reduction of general histone acetylation and NF-κB-dependent activation of the pro-apoptotic Bim promoter. To restore the normal acetylation levels of RelA and histones and obtain neuroprotection we combined the histone deacetylase (HDAC) inhibitor entinostat (MS-275) with the sirtuin-1 activator resveratrol.
The combined use of MS-275 and resveratrol, by restoring normal RelA acetylation, elicited a synergistic neuroprotection in neurons exposed to OGD. This effect correlated with MS-275 capability to increase total RelA acetylation and resveratrol capability to reduce RelA K310 acetylation through the activation of an AMP-activated protein kinase–sirtuin 1 pathway. The synergistic treatment reproduced the acetylation state of RelA observed in preconditioning ischemia. Neurons exposed to the combined drugs totally recovered the optimal histone H3 acetylation. In vivo studies in mice subjected to MCAO and treated with lowest doses of MS-275 (2 μg/kg) and resveratrol (68 μg/kg) confirmed the neuroprotective synergy. Both the infarct volume and the neurological deficits were significantly reduced by the drug association. Chromatin immunoprecipitation analysis of cortices harvested from treated mice showed that the RelA binding and histone acetylation increased at the Bcl-xL promoter and decreased at the Bim promoter.
In the present work we tested the combined effects of Resveratrol with the clinically used HDAC inhibitor, valproic acid (VPA).
Methods:
We used both in vitro and in vivo models of brain ischemia: oxygen glucose deprivation (OGD-3h) in primary cortical neurons and the transient middle cerebral artery occlusion (MCAO) in mice.
Results:
In primary neurons exposed to OGD the combined use of resveratrol and VPA, led to a synergistic neuroprotection. The post-OGD treatment of neurons with subthreshold concentrations of VPA (1uM) and resveratrol (3uM) reduced the cells death, likewise the application of single drugs at the maximal effective concentration (100 uM VPA, 30 uM resveratrol). The global H3 histone acetylation was reduced after OGD exposure and recovered in neurons treated with the synergistic combination of drugs (VPA 1 uM and resveratrol 3 uM). Western blot analysis of BDNF suggested that the treatment with resveratrol and VPA restored the protein expression, otherwise depressed by the OGD exposure.
In vivo results demonstrated that the treatment with an antiepileptic dose of VPA (20 mg/kg) or a neuroprotective dose of resveratrol (6800 μg/kg) in mice exposed to MCAO, significantly reduced the infarct volume, but not the neurological deficits. By contrast, treatment with the drugs in combination, even at doses 100-fold lower (VPA 200 ug/kg) and 10-fold lower (resveratrol 680 ug/kg), significantly reduced both the infarct volume and the neurological deficits.
We can conclude that likewise entinostat, VPA can elicit a synergistic neuroprotection with resveratrol in experimental models of post-ischemic brain injury. The synergistic effect relies on VPA doses 100 fold-lower than the antiepileptic ones.