Combination of PEA (palmitoylethanolamide) with luteolin promotes a synergistic neuroprotection and mast cells modulation in cell-based models of brain ischemia.

E. Parrella[1], V. Porrini[1, 2], R. Iorio[1], R. Piva[1], M. Benarese[1], M. Fusco[3], PF. Spano[1, 2], M.Pizzi[1, 2]

[1] Dept. of Molecular and Translational Medicine, University of Brescia, Italy
[2]IRCCS San Camillo, Venezia, Italy
[3]Epitech Group s.r.l. Saccolongo, Padova, Italy

Mast cells (MCs) are bone marrow derived perivascular resident cells characterized by a large number of dense granules containing histamine and heparin. MCs are widely distributed throughout all vascularized tissues, especially in anatomical regions that are directly exposed to the environment such as the skin and airways (Metz et al., 2007). Mastocytic activation occurs upon response to a wide range of physical and chemical stimuli and results in the release of inflammation mediators stored in the MC granules or synthetized *ex novo*. Initially known mainly for their pathogenic role in allergic and anaphylactic reactions, it is now well documented MCs involvement in processes of innate and adaptive immunity and inflammation. MCs are present also in the central nervous system (CNS) and their participation in various CNS pathological processes, including traumatic brain injury, multiple sclerosis and brain ischemia, is documented (Nelissen et al. 2013).

The aim of this study is to test the efficacy of PEA and luteolin, two epigenetic molecules potentially active on mast cells, on a cellular model of brain ischemia (oxygen glucose deprivation, OGD (Lanzillotta et al. 2012)).

First we evaluated the effect of OGD on viability and degranulation of MC/9 cells, cloned mastocytes derived from mouse fetal liver. Mastocytic degranulation was measured by toluidine staining and β-hexosaminidase release assay, cytotoxicity was assessed by measuring the amount of lactate dehydrogenase (LDH) released by the cells. OGD exposure promoted mast cell degranulation and LDH release in a time-dependent fashion, with the mastocytic activation becoming significantly different from control after six hours of OGD exposure. Next, we investigated whether OGD activated mast cells could provoke neuronal injury or amplify hypoxia induced neurotoxicity. Our results showed that incubation of primary mouse cortical neurons with conditioned medium derived from mast cells subjected to six hours of OGD induced significant neurotoxicity. Moreover, mast cells exacerbated neuronal damage in a co-culture neuron-MC/9 cell system exposed to six hours of OGD.

Next, we tested different concentrations of PEA and luteolin, alone or in association, on neurons or MC/9 cells exposed to hypoxic insult. Luteolin showed a mild neuroprotective activity when tested on neurons subjected to OGD, whereas PEA did not exhibit any effect at all. However, the association of the two drugs (1:10 and 1:100, luteolin:PEA) elicited a synergistic neuroprotection in cortical neurons exposed to OGD. The two molecules displayed a similar profile in modulating MCs activation upon OGD treatment. Luteolin, when used in the concentrations range active on neurons, reduced mastocytic activation and MC/9 LDH release, whereas no effect was observed for PEA. Again, when tested in association, the two compounds exhibited a synergistic modulatory activity on stimulated MCs, decreasing mastocytic degranulation and cytotoxicity. Finally, the toxicity observed on neurons incubated with conditioned medium derived from OGD-exposed MCs was abrogated by the pre-treatment of MC/9 cells with the association PEA-luteolin.

Taken together, our results strongly suggest that the association PEA-luteolin is endowed with a dual activity, neuroprotective on neurons and modulatory on mastocytic activation. These aptitudes make the association PEA-luteolin a promising treatment able to protect neurons from ischemic damage.

Lanzillotta et al. (2012), Neurobiol Dis 49C:177-189.

Metz et al. (2007) Immunol Rev 217:304-328.

Nelissen et al. (2013). Acta Neuropathol 125:637-650.