APOPTOTIC ENDOTHELIAL CELL DEATH INDUCED BY ROS IS REVERTED BY TREATMENT WITH BH4 PEPTIDE (SNRELVVDFLSYKLSQKGYS)

Cantara S., 1° anno di corso del Dottorato in Medicina Molecolare, XVII ciclo. Durata del Dottorato in anni: 3. Sede di servizio: Istituto di Scienze Farmacologiche, Università degli Studi di Siena, Via A. Moro 2, 53100 SIENA.

A reduction in cerebral blood flow is responsible for metabolic and functional diseases, and induces a neuronal and endothelial damage and cell death.
There are two different forms of cellular death: apoptosis and necrosis. Ischemic injury-induced cell death has traditionally been characterized as necrosis, however apoptotic neurons are found in the penumbra where injury is less severe and during reperfusion . Mitochondria may be important in the transmission of apoptotic signals during ischemia in order to induce caspase activation. There is strong evidence of caspase-3 activity in ischemic brain, which might be mediated by caspase-11, a caspase which is specifically induced by ischemic injury.
The BH4 domain of antiapoptotic Bcl-2 family members is responsible for the antiapoptotic activity of these proteins. Bcl-2 and Bcl-$_{xl}$ act by their BH4 domain in inhibit Cytocrom C release and the mithocondrial membrane potential loss.
The purpose of this project is to study the antiapoptotic effect of BH4 peptide on the endothelial cell exposed to stress conditions. ROS formation produced during ischemic injury is responsible for cell damage and apoptosis. To mimic this condition H_2O_2 has been used.
Apoptosis has been determinated by measuring Caspase-3 activity and by assessement of morphologic changes visualized by staining with 4'-6'-diaminidino phenylidole (DAPI) and Acridine Orange /Ethidium bromide (AO/EB).
Functional assays to measure cell growth (total cells number and DNA synthesis) have been performed.
BH4 peptide (SNRELVVDFLSYKLSQKGYS) has been synthesized in collaboration with Professor Philip E. Thorpe (Southwestern Medical Center, Dallas, Texas).
Different concentrations of H_2O_2 have been tested, 5uM of H_2O_2 did not induce cell death, while 20 – 30 uM induced cell loss.
When caspase-3 activity was mesaured, BH4 was effective in reducing apoptotic cells death. In proliferation assays, BH4 was able to protect from cell loss. Furthermore in condition when apoptosis was not induced, addition of BH4 peptide induced CVEC to proliferate (60% vs basal cell proliferation). All the protective effect exerted by BH4 peptide were not dose dependent since the lowest dose tested (0.1 nM) as well as the highest one (10 nM) produced similar results.
In conclusion our results indicated that addition of BH4 peptide to cultured endothelial cells can prevent ROS – induced apoptosis and cell death.