Autophagy protects neurons and astrocytes from bilirubin-induced cytotoxicity

Mohammed Qaisiya[1], Paula Mardesic[2], Claudio Tiribelli[2], Beatrice Pastore[3] and Cristina Bellarosa[2]

[1]Hebron University, Palestine

[2]Fondazione Italiana Fegato Onlus, Italy

[3]International School for Advanced Studies, Italy

Unconjugated Bilirubin (UCB) neurotoxicity involves oxidative stress, calcium signaling and ER-stress. The same insults also induce autophagy, a process of self-eating, with both a pro-survival and a pro-apoptotic role. Our aim was to study the outcome of autophagy activation by UCB in the highly sensitive neuronal SH-SY5Y cells and in the resistant astrocytoma U87 cells. Upon treatment with a toxic dose of UCB, the conversion of LC3-I to LC3-II was detected in both cell lines. Inhibition of autophagy by E64d before UCB treatment increased SH-SY5Y reduction of cell viability from 40% to 60% and made U87 cells sensitive to UCB. In SH-SY5Y cells autophagy related genes ATG8 (5 folds), ATG18 (5 folds), p62 (3 folds) and FAM 129A (4.5 folds) were induced 8h after UCB treatment while DDIT4 up-regulation (13 folds) started at 4h. mTORC1 inactivation by UCB was confirmed by phosphorylation of 4-EBP1. UCB induced LC3-II conversion was completely prevented by pre-treating the cells with the calcium chelators BAPTA and reduced by 65% using the ER-stress inhibitor 4-PBA. Pre-treatment with the PKC inhibitor reduced LC3 mRNA by 70% as compared to cells exposed to UCB alone. Finally, autophagy induction by Trifluoroperazine (TFP) increased the cell viability of rat hippocampal primary neurons upon UCB treatment from 60 to 80%. In SH-SY5Y cells, TFP pre-treatment blocked the UCB-induced cleaved caspase-3 protein expression, decreased LDH release from 50 to 23%, reduced the UCB-induction of HO1, CHOP and IL-8 mRNAs by 85%, 70% and 97%. Collectively these data indicate that the activation of autophagy protects neuronal cells from UCB cytotoxicity. The mechanisms of autophagy activation by UCB involve mTOR/ER-stress/PKC/calcium signaling.