The role of autophagy in alcoholic cardiomyopathy *in vitro*

J. Maiuolo[1], A. Maretta[1], F. Oppedisano[1], M. Gliozzi[1,2], C. Carresi[1], V. Musolino[1], A. Scarcella[1], C. Giancotta[1], F. Lauro[1,2], L.A. Giancotti[1,2], S. Ilari[1,2], C. Morabito[1,2], F. Scarano[1], E. Palma [1], C. Muscoli [1,2], V. Mollace [1,2]

[1]Interregional Research Centre for Food Safety & Health, Dept. of Health Sciences, University 'Magna Graecia' of Catanzaro, Catanzaro, Italy
[2]San Raffaele IRCCS, Rome, Italy

Alcoholism is one of the most etiologic identified factor in heart disease. Chronic ethanol consumption for long periods is the main cause of a progressive cardiac dysfunction and it can determine a particular cardiac disease known as Alcoholic CardioMyopathy (ACM) by direct damage to the myocardial structure. Chronic ethanol exposure induces cardiac remodeling and cardiomyocyte hypertrophy. Numerous clinical and experimental evidence have documented that prolonged intake of high alcohol amounts (more than 5 units per day) is able to induce lesions of the heart muscle, while the daily intake of moderate alcohol, about 1-2 glasses per day, may reduce the risk of developing a heart failure or myocardial infarction. Cellular damage induced by xenobiotics exposure determines a cytoprotective mechanism to maintain cellular homeostasis and to revert cellular stress. Autophagy is an important cellular process that contributes to cell recovery. Many xenobiotics are known to modulate the induction or the rate of autophagy.
We conducted our experiments on a cell line of cardiomyocytes. In particular H9c2 cells were treated with different concentrations of ethanol (1, 100, 500 and 1000 μM) for different times (3, 6, 9 and 12 days). Through experiments of cell viability by MTT assay and Trypan blue assay, we found that treatment with ethanol (concentrations and times considered) did not determine cell death. However, we wondered whether the treated cells with ethanol perceived or not a suffering condition. This hypothesis was tested by experiments in which cells were co-treated with ethanol and together with methylmercury or doxorubicin. Methylmercury and doxorubicin were used at concentrations that are not normally toxic to our cells (5 and 1 μM respectively for 24 hours). In particular, the co-treatment of EtOH 1000 μM for 12 days with methylmercury 5 μM or with doxorubicin 1 μM resulted in an increased mortality of 6 and 7 times respectively ifcompared to untreated cells. The results obtained were statistically significant. We then evaluated whether autophagy was involved or not and the expression of the protein Beclin 1 was determined under our experimental conditions. Treatment with EtOH 500 and 1000 μM for 12 days resulted in an increased expression of Beclin 1 of 1.5 and 2 times, respectively, compared to the control. The co-treated cells with EtOH 1000 μM for 12 days and methylmercury or doxorubicin have shown an expression of Beclin 1 greater than the control but lower compared to the same single treatment. These preliminary data suggest that chronic and continous ethanol consumption promotes the development of a form of autophagy presumably protective and that the damage is not yet clear but becomes real after low and not toxic doses of methylmercury or doxorubicin.

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