OXALOMALATE INDUCES FERRITIN BIOSYNTHESIS AND PROTECTS CELLS FROM OXIDATIVE STRESS

Irace C., 2° anno di corso del Dottorato in Scienza del Farmaco, XVI ciclo. Durata del Dottorato in anni: 3. Sede di servizio: Dipartimento di Farmacologia Sperimentale, Facoltà di Farmacia, Via D. Montesano 49, Università "Federico II", 80131-Napoli**.**

Free iron can catalyse reactions which generate reactive oxygen free radical species that lead to proteins, lipids and DNA damage. To prevent these deleterious effects, cells have evolved homeostatic mechanisms regulating transport, storage and mobilization of cellular iron. Ferritin, the major iron storage protein, is formed by subunits of two types (H and L) and it is capable of binding as many as 4500 atoms of this metal. Its ability in sequestering iron gives ferritin the dual functions of detoxification and iron reserve. Expression of this protein is primarily regulated, at translational level, through specifically regulated protein-RNA interactions between iron regulatory proteins (IRP1 and IRP2) and iron-responsive elements (IREs) contained within the 5'-UTR of the H- and L-ferritin mRNA. IRP1 has been identified as the cytosolic counterpart of mitochondrial aconitase and it is a bifunctional protein that switches from the 4Fe-4S aconitase form, with enzymatic activity, to IRP1 form with IRE-binding activity, in response to cellular iron level and to reductant/oxidant substrates.
It has been previously reported by our research group that oxalomalate (OMA, α-hydroxy-β-oxalosuccinic acid), a competitive inhibitor of aconitase, remarkably decreases the IRE-binding activity of IRP1 on different cell types. OMA, by an interaction with IRP1, inhibits the IRP-IRE binding of ferritin mRNAs; consequently we can hypothesise that the translation of both H- and L-ferritin could be increased.
In an attempt to test this hypothesis, we have incubated mouse 3T3-L1 pre-adipocytes with OMA for different times and then we analysed the IRE-binding activity of IRPs by electrophoretic mobility shift assay. The obtained results showed that OMA inhibits the RNA-binding activity of IRP1 with a remarkable decrease after 12h of OMA exposure, afterwords the binding activity slightly increased, returning at basal levels.
To evaluate the OMA effect on intracellular ferritin levels, we have analysed, by Western-Blot method, lysates derived from mouse 3T3-L1 pre-adipocytes, mouse 3T3-NIH fibroblasts, rat C6 glioma cells and human SH-SY5Y neuroblastoma cells cultured with OMA. The results clearly demonstrate that the decrease in IRP1 binding-activity OMA-induced leads to a significative increase in the intracellular level of ferritin.
Considering that IRPs are subjected to regulation by additional iron-indipendent signals, including oxidative stress, we can also predict that OMA could protect the IRP1/aconitase from the oxidative stress induced by H_2O_2. Experiments performed *in vivo* and *in vitro* showed that OMA, through a molecular specific interaction with the active site of aconitase/IRP1 protein, protects the Fe-S cluster of aconitase form from the H_2O_2 oxidant attack.
Finally, we evaluated lipid peroxidation products, assessed by quantification of thiobarbiturate-reactive material, in cells cultured with or without OMA and exposed to iron challenge. We observed that overexpression of ferritin OMA-induced is capable to prevent the iron-dependent cellular lipid peroxidation and protect cell against oxidative stress.