**Graphene oxide and cytotoxicity studies in neuroblastoma cell lines**

Graphene derives from graphite and is a single layer of carbon atoms arranged in a honeycomb two- dimensional (2-D) crystal. It exhibits excellent chemical-physical, biochemical, mechanical and engineering properties, extremely useful for several applications including drug delivery.

Graphene oxides are easier to functionalize as they have free COOH and OH groups that can be used as binding sites for multiple bio molecules, the physical form of GO is also an issue because it may have toxic or less toxic properties. We are showing here that differently synthesized GO exhert various degrees of cytotoxicity in different cell lines.

GO nanoribbons, obtained by oxidative unzipping of commercial single wall carbon nanotubes (Sigma) and GO nanosheets, synthesized by electrochemical exfoliation of graphite (patent N 102015000023739, Tor Vergata University), were analyzed in 2 neuroblastoma cell lines. This synthesized GO is known as “green” for its low environmental impact due to absence of metals and untoxic extraction method.

Neuroblastoma is the most common solid neoplasia in children. The hallmark of these tumors is the high number of different clinical variables, ranging from highly metastatic, rapid progression and resistance to therapy to spontaneous regression or change into benign ganglioneuromas. Patients with neuroblastoma are grouped into different risk groups that are characterized by different prognosis and different clinical behavior. Relapse and mortality in high risk patients is very high in spite of new advances in chemotherapy.

In our experimental setting, cells were exposed to low doses of GO for different times in order to investigate whether GO was a good vehicle for biological molecules delivering individualized therapy. Cytotoxicity in both cell lines was studied by measuring cellular oxidative stress (ROS), mitochondria membrane potential, expression of lysosomial proteins and cell growth. GO uptake and cytoplasmic distribution of particles were studied by Transmission Electron Microscopy (TEM) for up to 72 h. The results show that GO at low concentrations increased ROS production and induced autophagy in both neuroblastoma cell lines within a few hours of exposure, events that, however, are not followed by growth arrest or death. This set of experiments showed that exposure times and GO concentration are crucial for cytotoxicity. Another issue to be addressed in the study of nanomaterials is the persistence of reagents used for their extraction in the human body and in the environment.

For this reason, “green” GO was selected as a possible carrier of an anticancer drug, the S29 *(1-(2-chloro-2-(4chlorophenyl)ethyl)-N-(4-fluorobenzyl)-1H-pyrazolo[3,4-d]pyrimidin-4- amine),* inhibitor of a cytoplasmic tyrosine kinase (c-SRC). S29 is able to reduce tumour mass in neuroblastoma but it shows an unfavorable pharmacokinetic profile. We have shown that when S 29 is combined with GO, exerts higher antitumoral effects than when administerd alone and in conclusion we may say that GO used at the lowest concentration could be a promising nanomaterial for delivering small interfering molecules in the tumor site.