A NEW SYNTHETIC ISOTHIOCYANATE DERIVATIVE (MG28) AS AN IMMUNOGENIC CELL DEATH INDUCER: IN VITRO CHARACTERIZATION OF ITS PHARMACO-TOXICOLOGICAL PROFILE.

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The immunogenicity of malignant cells has recently been recognized as a critical determinant of efficacy in cancer therapy. Some anticancer agents, such as mitoxantron, are able to induce a peculiar form of cell death characterized by an increased immunogenic potential. So far, the most potent inducers of immunogenic cell death (ICD), such as the hypericin-photodynamic therapy, elicit danger signalling through direct oxidative-endoplasmic reticulum (ER) stress (Krysko et al., 2012).

Isothiocyanates (ITCs) are naturally occurring small molecules that are formed from glucosinolate precursors of cruciferous vegetables. Many ITCs, both natural and synthetic, display anticarcinogenic activity because they reduce activation of carcinogens and increase their detoxification. Recent studies show that they exhibit anti-tumor activity by affecting multiple pathways including apoptosis, MAPK signaling, oxidative stress, and cell-cycle progression (Fimognari and Hrelia, 2007).

The aim of this study was to characterize the cytotoxic, genotoxic and proapoptotic potential and the capability to induce oxidative and ER stress of a newly synthesized compound, MG28. This molecule consists of an ITC moiety fused to a renowned ER transporter, capable to direct the active group straight to the ER (Meinig et al., 2015). Literature data about the chosen ER transporter support the hypothesis that it passively diffuses through the plasma membrane, reaches the ER and, there, is metabolized by ER carboxylesterases and frees the active moiety (Hakamata et al., 2014).

The research was performed on a human lymphoblastoid cell line (Jurkat) and a human epithelial cervix carcinoma cell line (HeLa). On HeLa we localized MG28, while on Jurkat we characterized its antitumor and genotoxic profile. MG28 induces a dose-dependent decrease in the number of viable cells and the IC50 was reached at 5.31 µM. MG28 triggers the apoptotic process, as demonstrated by the fast halo alkaline assay at non-denaturing conditions and by the activation of caspase 3. To determine whether the apoptosis was mediated through the death receptor or the mitochondrial pathway, the activity of caspase 8 and the alteration of the mitochondrial potential were investigated. Caspase 8 activity increased dose-dependently after treatment with MG28 and at the highest tested concentration it induced a 2.2 fold increase versus untreated cells. The fraction of cells with decreased mitochondrial potential reached around 33% at the highest tested concentration versus 5.3% of untreated cells. Taken together, these data suggest that MG28 induces apoptosis through both pathways. Then, to assess whether MG28 could be an ICD inducer, we firstly proved its localization in the ER. HeLa were transfected with an ER-targeted red fluorescent protein and then treated with MG28 at subtoxic concentrations. The microscopic

analysis showed a complete, but not selective, MG28 localization in the ER. Oxidative stress is another prerequisite for ICD induction and MG28 increased intracellular ROS levels. Since oxidative stress can cause DNA damage, we investigated if MG28 was genotoxic. MG28 did cause DNA double strand breaks, but not fully due to ROS-increased levels. In fact, Jurkat pretreatment with buthionine sulfoximine, an inhibitor of the antioxidant glutathione de novo synthesis, didn't increase the genotoxic insult. More, preliminary results obtained through a plasmid cleavage assay, revealed that MG28 caused direct DNA damage.

Our findings prove that MG28 is a conceivable candidate for ICD induction studies. Overall, with the aim to improve therapeutic outcomes of anticancer drugs through the involvement of immune system, the conjugation of active natural compounds with ER transporters represents a promising pharmacological strategy in the oncological area.

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