Cytotoxic Activity of Dendrimer Nanoparticles and Dendrimer Drugs Formulations on Human Neuroblastoma Cells: Our Recent Update

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Background

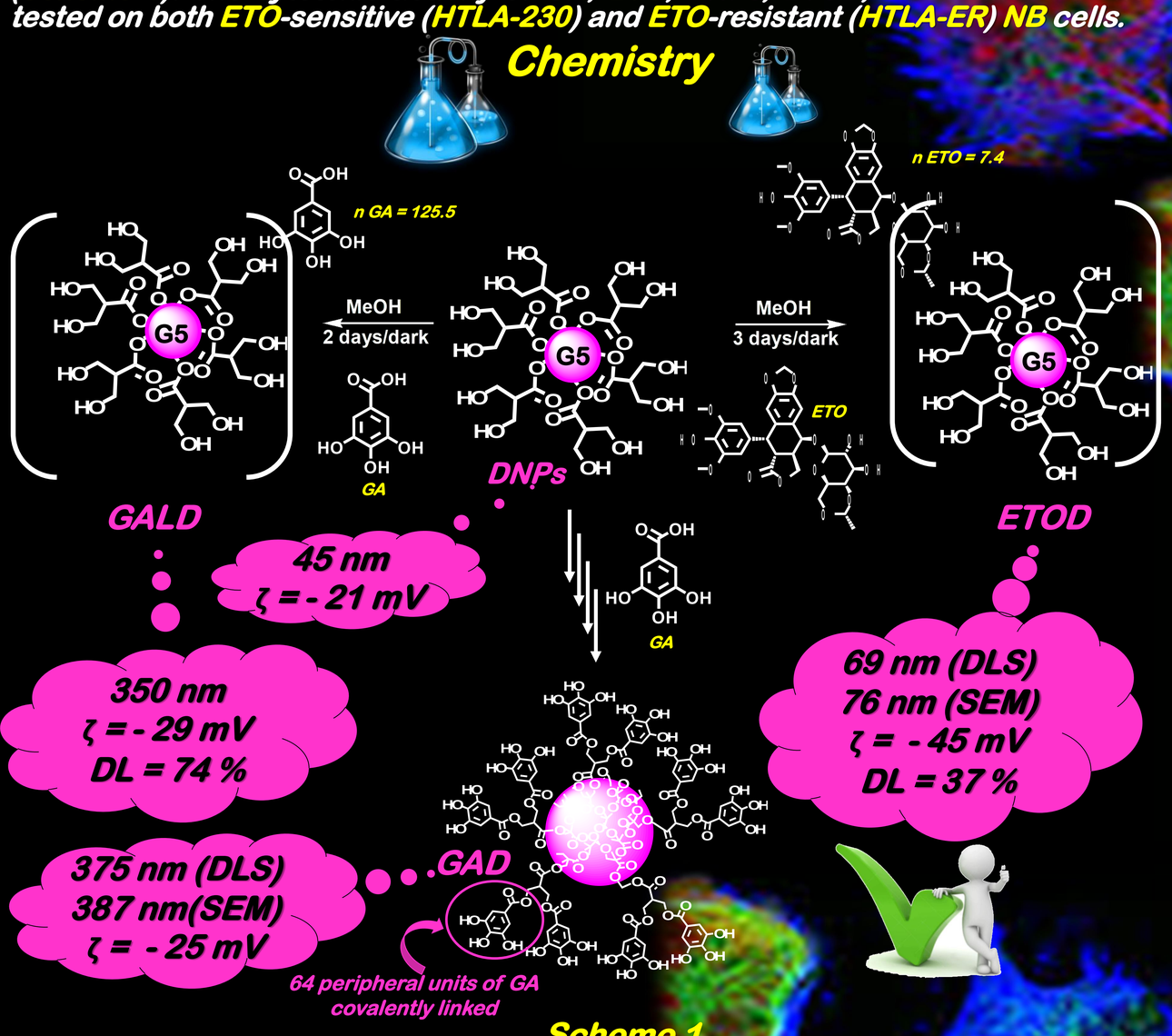
Human neuroblastoma (NB) is a pediatric tumor, that usually develops resistance after an initial response to therapy. Etoposide (ETO), which is a drug commonly used to treat NB, exerts anticancer effects by increasing the generation of reactive oxygen species (ROS) [1,2]. Similarly, gallic acid (GA) has shown to induce ROS-dependent death in human prostate cancer cells [3] and to exert apoptotic effects in several kind of tumors, associated with low toxicity to healthy cells [4,5]. Unfortunately, the low stability, poor solubility and the unfavorable pharmacokinetic profile of ETO and GA, negatively affect their therapeutic efficacy [1,2].

Drug Delivery Systems (DDSs), deriving from the application of engineered nanotechnology, represent reliable tools for obtaining a controlled temporal and spatial drug delivery also of poorly water-soluble drugs, for improving their stability and therapeutic efficacy, as well as for reducing dosages, frequency of administration and systemic toxicity. In this regard, dendrimers have for years been the emerging polymer architectures known for their defined structures, versatility in drug delivery and high functionality.

Our Recent Studies

In order to obtain new and more favorable formulations of GA and ETO, biodegradable dendrimer nanoparticles (DNPs) were used to trap ETO [2], as well as to encapsulate and covalently bind GA [1], thus obtaining one dendrimer formulation loaded with ETO (ETOD) and two other ones loaded with GA, i.e. GALD where GA was physically entrapped and GAD, where it was covalently linked (Scheme 1). The cytotoxic activity of GA, ETO, DNPs, ETOD, GALD and GAD was tested on both ETO-sensitive (HTLA-230) and ETO-resistant (HTLA-ER) NB cells.

Chemistry



Scheme 1

Experiments

Cell viability and ROS production in selected NB cells populations, treated with free GA, free ETO, DNPs, ETOD, GALD and GAD, were studied to establish their anticancer properties. The results have been reported in Figure 1, 2 , 3 and 4.

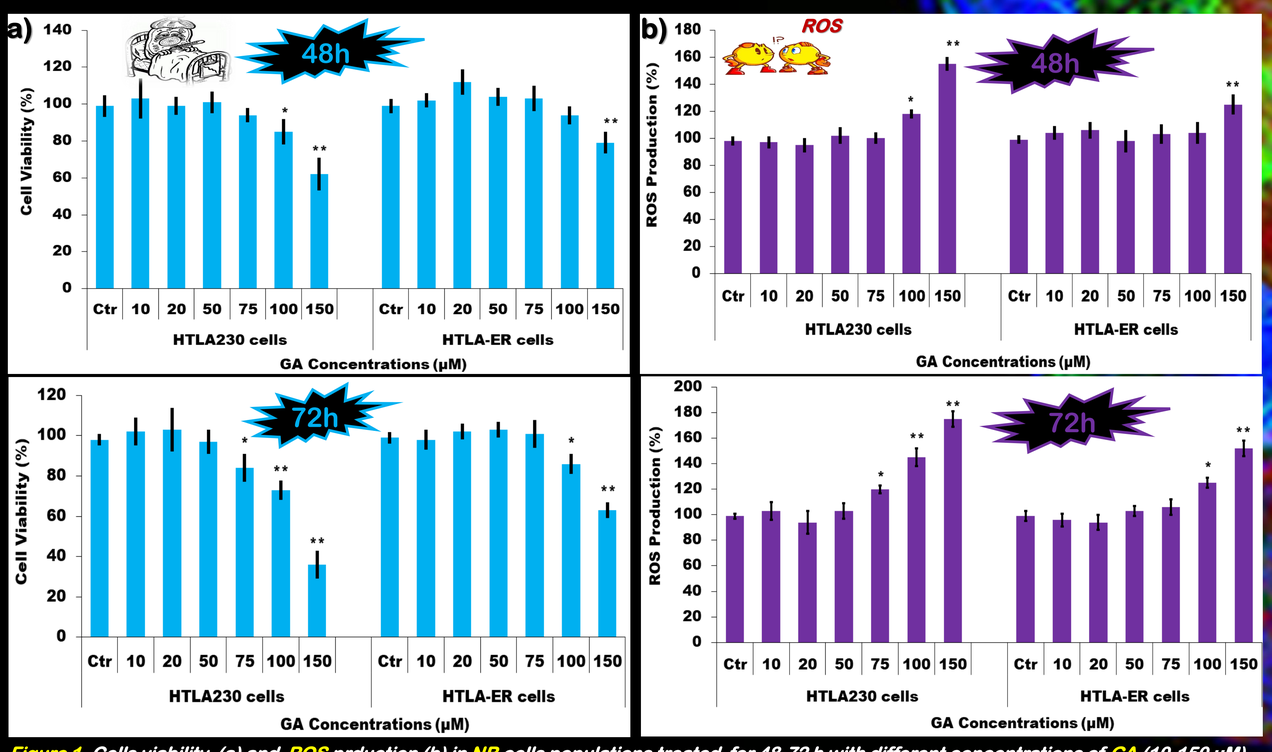


Figure 1. Cells viability (a) and ROS prduction (b) in NB cells populations treated for 48-72 h with different concentrations of GA (10-150 μM) .

Results

As for GA, tested for the first time against NB cells, time-course and dose-dependent experiments were performed on HTLA-230 and HTLA-ER cells (Figure 1). As for ETO, ETOD, DNPs, GALD and GAD, they were used in the concentrations explained in the captions of Figure 2, 3 and 4. A significant reduction in cell viability (a) and a correlated significant improvement of ROS production (b) was observed at very high concentrations of GA (100-150 μM) for both cells populations (Figure 1). On the contrary, concentrations of GA in the range of 10-75 μM were totally ineffective. Unexpectedly, DNPs, essayed for the first time as an anticancer device, proved a ROS-induced significant cytotoxic activity similar to that of ETO, but at a concentration 7 times lower. ETOD showed a time-dependent synergistic behavior between ETO and DNPs, which led to a reduction in cells viability of 65 % and to an increase of ROS production of 190 %, after 72 h of exposure. This scenario makes thought to a low and protracted release of ETO along time.

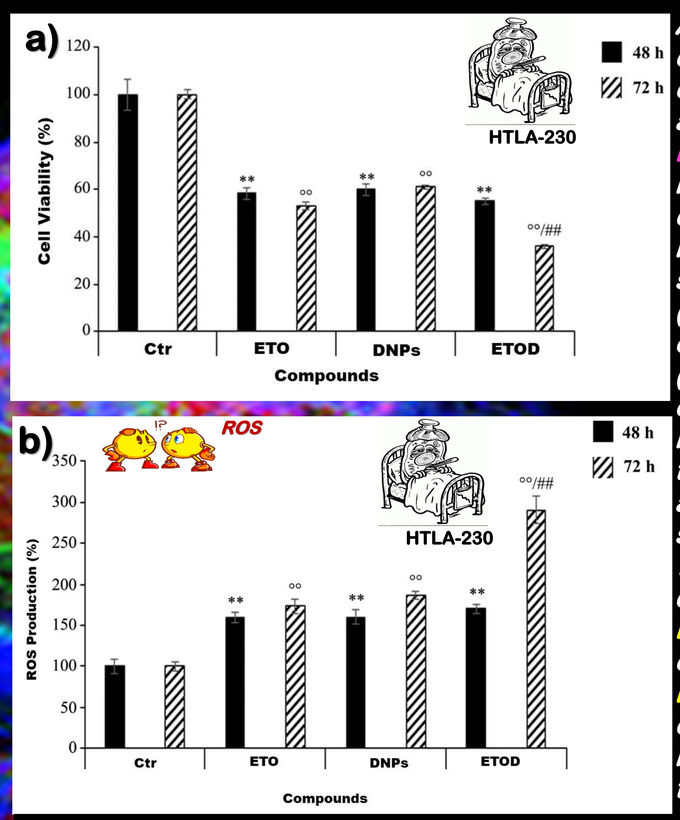


Figure 2. Cells viability (a) and ROS prduction (b) in RTLA-230 NB cells treated for 48-72 h with ETO at the maximum concentration used in clinical administrations (1.25 μM), with ETOD in a concentration able to deliver ETO 1.25 μM, and with DNPs in the concentraion delivered by the amount of ETOD used (0.169 μM).

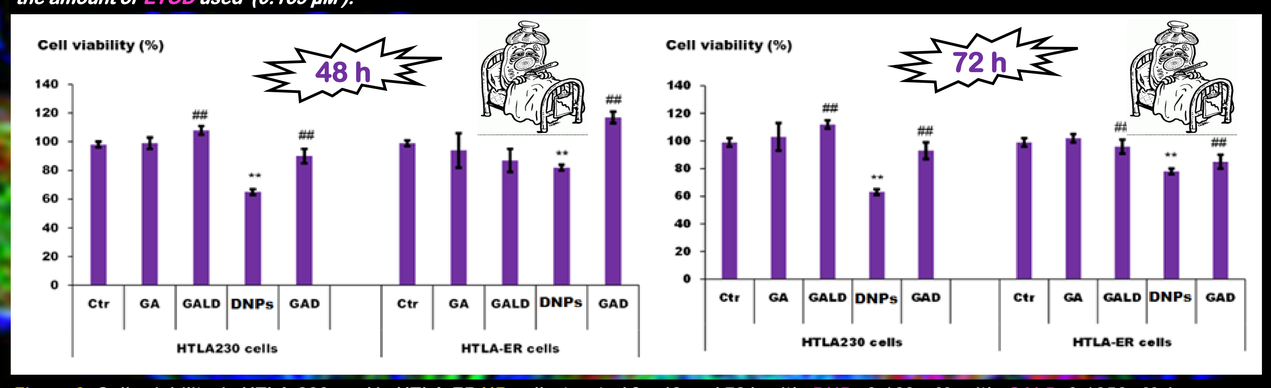


Figure 3. Cells viability in HTLA-230 and in HTLA-ER NB cells treated for 48 and 72 h with DNPs 0.169 μM, with GALD 0.1656 μM, i.e. a concentration able to provide DNPs 0.169 μM and GA 21.20 μM, and with GAD 0.3316 μM , which is the concentration able to deliver 21.20 μM GA.

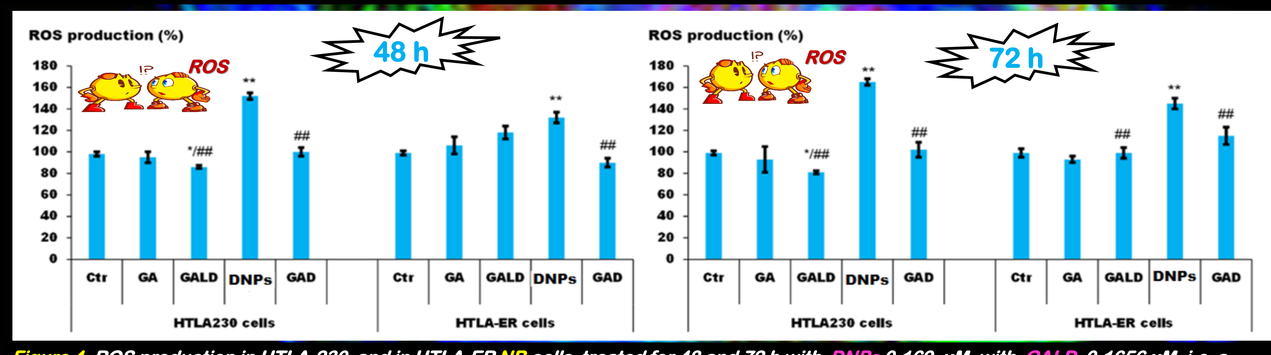


Figure 4. ROS production in HTLA-230 and in HTLA-ER NB cells treated for 48 and 72 h with DNPs 0.169 μM, with GALD 0.1656 μM, i.e. a concentration able to provide DNPs 0.169 μM and GA 21.20 μM, and with GAD 0.3316 μM , which is the concentration able to deliver 21.20 μM GA.

Regarding the experiments on GALD and GAD, the results were reported in Figure 3 (cell viability) and in Figure 4 (ROS production). DNPs, administered at the same active concentration of 0.169 μM, confirmed their potent pro-oxidant, ROS-dependent cytotoxic activity on HTLA-230, and also established their efficacy on NB HTLA-ER cells. As expected, GA administered at the low concentration adopted (21.20 μM) did not influence significantly either cell viability and ROS generation of both cell populations. The results obtained with the GA dendrimer formulations suggested that also GA delivered by the amounts administered of GALD and GAD (21.20 μM) displayed a similar behavior. Curiously, the nanoparticulate GA present in GALD and GAD was capable to exert an antioxidant activity that nullified the strong pro-oxidant activity observed for empty DNPs in both cell populations, at very low doses and both after 48 and 72 hours of exposure.

Conclusions

These results suggest that promising new strategies can be developed for the treatment of cancer, by applying nanotechnology to available and commonly adopted drugs such as ETO and to natural compounds, such as GA. Free GA demonstrated a dose-dependent ROS-mediated cytotoxicity both on NB cells sensitive and resistant to ETO, but interestingly, when administered in dendrimer formulations, at a dose not cytotoxic to NB cells, it nullified any pro-oxidative activity of DNPs. Taken together, DNPs could represent a key to develop new devices against NB, while ETOD could be a biodegradable device for efficient and protracted delivery of ETO into NB cells. GALD and GAD, due to the presence of GA, were inactive on NB cells, but GA, when scaled into nanoparticles, even at a very low dose, showed a remarkable ability to counteract the production of ROS induced by DNPs, thus making conceivable its possible use as protective agent towards healthy cells .

1. Alfei, S.; Marengo, B.; Zuccari, G.; Turrini, F.; Domenicotti, C. Dendrimer Nanodevices and Gallic Acid as Novel Strategies to Fight Chemoresistance in Neuroblastoma Cells. *Nanomaterials* **2020**, *10*, 1243. 2. Alfei, S.; Marengo, B.; Domenicotti, C. Polyester-Based DendrimeNanoparticles Combined with Etoposide Have an Improved Cytotoxic and Pro-Oxidant Effect on Human Neuroblastoma Cells. *Antioxidants* **2020**, *9*, 50. 3. Russell, L.H., Jr; Mazzio, E.; Badisa, R.B.; Zhu, Z.-P.; Agharahimi, M.; Oriaku, E.T.; Goodman, C.B. Autoxidation of gallic acid induces ROS-dependent death in human prostate cancer LNCaP cells. *Anticancer. Res.* **2012**, *32*, 1595–1602. 4. Sun, G.; Zhang, S.; Xie, Y.; Zhang, Z.; Zhao, W. Gallic acid as a selective anticancer agent that induces apoptosis in SMMC-7721 human hepatocellular carcinoma cells. *Oncol. Lett.* **2016**, *11*, 150–158. 5. Aborehab, N.M.; Osama, N. Effect of Gallic acid in potentiating chemotherapeutic effect of Paclitaxel in HeLa cervical cancer cells. *Cancer Cell. Int.* **2019**, *19*, 154–154.

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