65P - Oxidative stress associated with Cu2+ to Cu+ reduction for eradicating wild type and multidrug resistant tumor cells

Sergey Tsymbal[1], Nicole Agadzhnyan[1], Alexander Shtil[1,2]
[1] ITMO University, Saint-Petersburg, Russia (*zimbal@scamt-itmo.ru)*
[2] Blokhin National Medical Research Center of Oncology, Moscow, Russia

Background

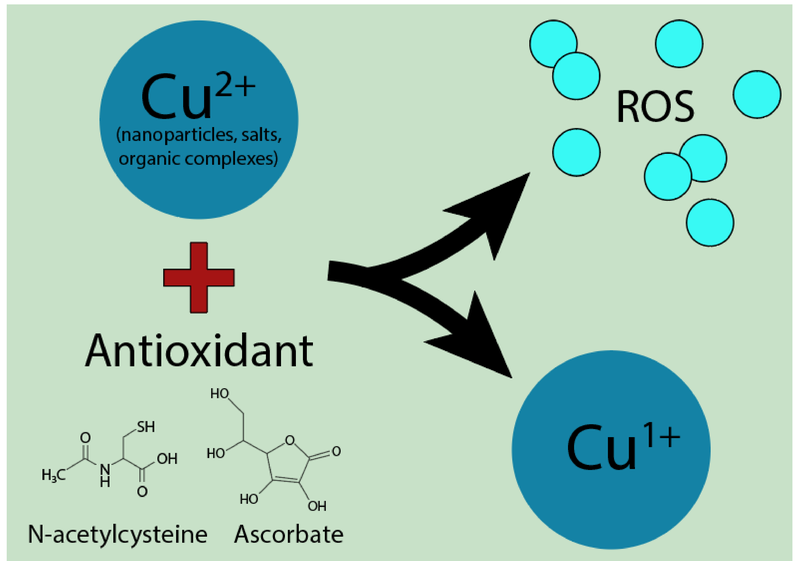
Tumor relapse often results from acquired drug resistance after a course of chemotherapy. In this case, the effectiveness of conventional or targeted drugs could be greatly reduced due to mutations, specific transporters, enhanced DNA repair, or disrupted cell death mechanisms. At the same time, cancer cells could be sensitive to the shift of the redox balance. Simultaneous addition of copper and antioxidants cause rapid reactive oxygen species (ROS) generation and cell death, which is promising way to deal with multidrug resistant cancer cells.

Methods

The panel of cell lines included HCT116 colon and MDA-MB-231 triple negative breast carcinomas as well as K562 (CML) cell line and its multidrug resistant K562/4 subline.

CuO NPs (80±20 nm determined by dynamic light scattering) were synthesized by the precipitation method. The cytotoxicity of copper compounds and NAC alone as well as their combinations was determined in MTT assays. Flow cytometry was used to analyze the mechanisms of cell response. Caspase-3 and poly(ADPribose) polymerase (PARP) were detected by immunoblotting.

Cytotoxicity of CuO NPs, copper acetate and copper organic complexes was greatly potentiated by antioxidants (N-acetylcysteine or ascorbate) for all tested cell lines after 72 h. The IC_{50} of the combination was 2-3 orders of magnitude smaller compared to copper compounds alone (Figure 1). Rapid cell death occurred within several hours after addition of the combination (Figure 2). ROS generation preceded propidium staining (Figure 3) suggesting oxidation as the main source of damage. Apoptosis was independent of caspase-3 or PARP cleavage (Figure 4). Importantly, the combinations of copper compounds with NAC were equally potent for K562 cells and the multidrug resistant K562/4 subline. Experiments in cell free systems revealed that reduction of Cu^{2+} to Cu^{+} upon interaction with the thiol group in NAC can trigger ROS formation (Scheme 1).



Scheme 1. Representation of ROS generation mechanism occurred upon copper reduction by the antioxidants

Results

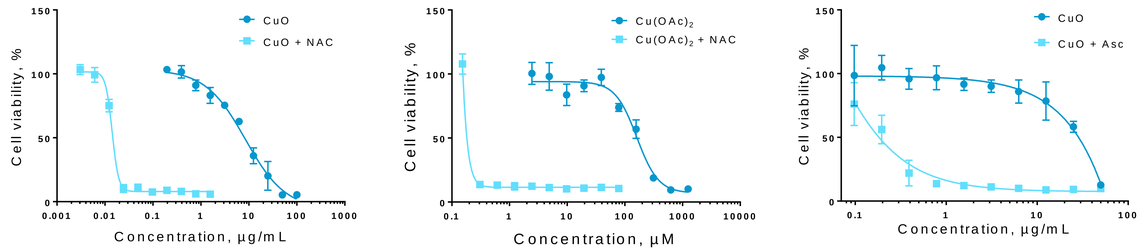


Figure 1. N-acetylcysteine (NAC) and ascorbate (Asc) dramatically increase the cytotoxicity of copper compounds

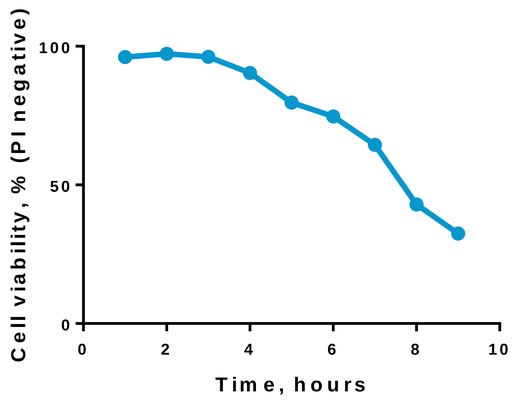


Figure 2. CuO + NAC provoke rapid cell death

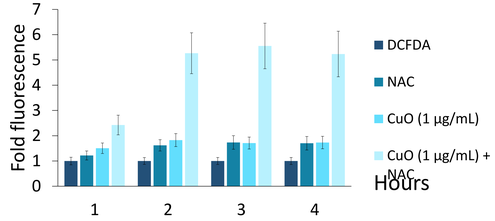


Figure 3. ROS measurements

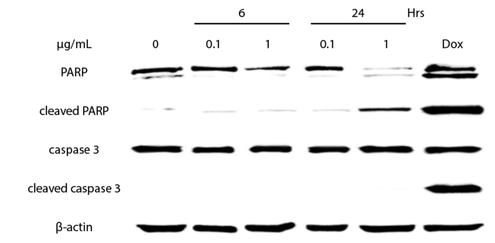


Figure 4. Immunoblotting on K562 cell line

Conclusions

Drug combinations strongly decreases viability of human tumor cell lines including the multidrug resistant counterparts. Rapid ROS generation caused by a reduction of divalent to monovalent copper can be promising for the purge of abdominal or thoracic cavities from metastatic cells or in situations when tumor cells acquire multidrug resistance during treatment.