

Abstract # C11

The oxidative stress inducer elesclomol requires copper chelation for its anticancer activity.

Masazumi Nagai, Nha Vho, Elena Kostik, Suqin He, Jane Kepros, Luisa S. Ogawa, Takayo Inoue, Ronald K. Blackman, Yumiko Wada, James Barsoum

Synta Pharmaceuticals Corp., Lexington, MA

Introduction: Elesclomol [N-malonyl-bis (N'-methyl-N'-thiobenzoyl hydrazide)] is a first-in-class investigational drug, believed to exert anticancer activity through the elevation of reactive oxygen species (ROS) levels leading to the activation of the mitochondrial apoptosis pathway. The mechanism of ROS induction by elesclomol was previously unknown. Data presented here shows that ROS is generated via chelation and redox cycling of copper.

Methods: Formation of a Cu chelate of elesclomol was analyzed by LCMS and single crystal X-ray diffraction. ROS was measured using DCF-DA and cytotoxicity assessed using a WST-8 assay primarily in Ramos human B cell lymphoma and M14 human melanoma cell lines. Cellular levels of free elesclomol and elesclomol-Cu were determined by LCMS. Redox potential was analyzed by cyclic voltammetry.

Results: Elesclomol readily formed a Cu chelate and strongly preferred Cu over zinc, iron or manganese in competition assays. Cu bound elesclomol in a 1:1 molar ratio and the chelate formed a flat rigid structure. The donation of four lone-pair electrons from elesclomol to Cu(II) yields higher hydrophobicity, which may facilitate greater cell permeability relative to free elesclomol. We evaluated the effect of Cu on both cellular uptake and cytotoxic activity of elesclomol on cultured cancer cells. Elesclomol lost cytotoxicity when applied to cells under Cu-starved conditions. The presence of Cu was required for elesclomol entry into cells. The cell membrane impermeable Cu chelator BCP blocked both uptake of elesclomol and cytotoxicity, indicating that elesclomol obtains Cu outside the cell and requires it for cellular entry and cytotoxicity. Elesclomol was able to obtain Cu from serum as well as from purified ceruloplasmin, the primary Cu-binding protein in blood. Elesclomol had poor activity against densely-plated cancer cells in culture, but became highly potent when extra Cu was added to the medium, indicating that Cu may be limiting under dense culture conditions. We next evaluated the impact of Cu on ROS generation. A cell-free assay system showed that elesclomol-Cu(II) was capable of efficient generation of ROS via the reduction of Cu(II) to Cu(I). A correlation was observed between redox potential and ROS production for Cu chelates of elesclomol and its analogs. Elesclomol also chelated nickel. However, while elesclomol-Ni was highly cell permeable, it was inactive for ROS production and cancer cell cytotoxicity. The lack of activity of elesclomol-Ni can be explained by its low redox potential. The redox potential of elesclomol-Cu(II) was -333mV, while that of elesclomol-Ni was -1100mV.

Conclusion: Elesclomol chelates Cu outside of cells and enters cells as elesclomol-Cu(II). At present, our data support the hypothesis that elesclomol generates ROS by redox cycling of Cu(II) to Cu(I), and that this process is necessary for its anticancer activity.