

The Oxidative Stress Inducer Elesclomol Requires Copper Chelation for its Anticancer Activity

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Abstract

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.

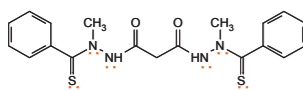
Results: Elesclomol readily formed a Cu chelate and strongly preferred Cu over zinc, iron or manganese in competition assays. Cu bound elesclomol in a 1:1 molar ratio and the chelate formed a flat rigid structure. The donation of four lone-pair electrons from elesclomol to Cu(II) yields higher hydrophobicity, which may facilitate greater cell permeability relative to free elesclomol. We evaluated the effect of Cu on both cellular uptake and the 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.

Introduction

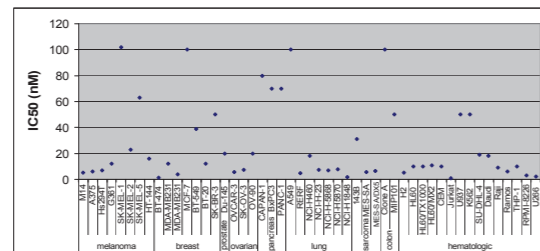
Elesclomol is a first-in-class investigational drug believed to exert anticancer activity through the elevation of reactive oxygen species (ROS) levels leading to the activation of the mitochondrial apoptosis pathway (Mol Cancer Ther 2008;7:2319-27). The mechanism of ROS induction by elesclomol was previously unknown. Data presented here shows that ROS is generated via chelation and redox cycling of copper.

Figure 1. Structure of elesclomol



Symmetrical structure including six lone pairs of electrons (•••) allows elesclomol to chelate metal ion

Figure 2. Elesclomol has nanomolar potencies in a broad range of cancer cell lines



Elesclomol has a median IC50 of 12 nM.

Figure 3. Elesclomol forms a chelate only with copper in competition with major transition metal ions

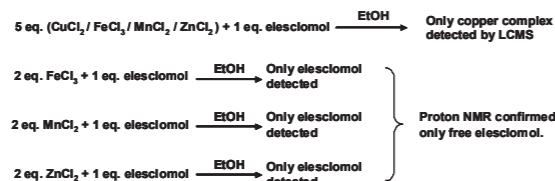
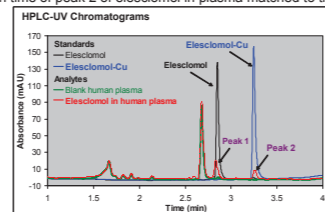


Figure 4. Elesclomol forms a copper chelate in human plasma

Retention time of peak 2 of elesclomol in plasma matched to that of elesclomol-Cu



Spectrophotometry identified plasma elesclomol-peak 2 as Elesclomol-Cu

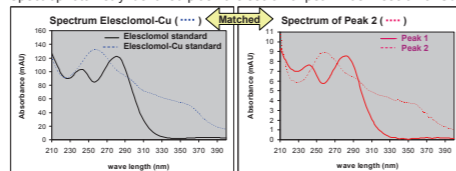
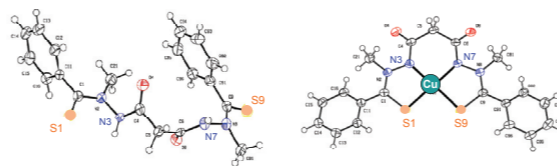


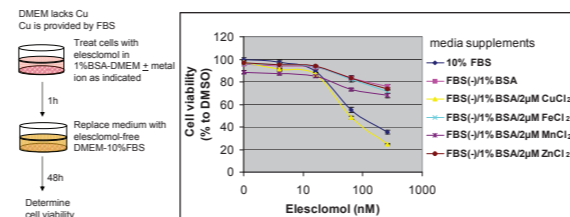
Figure 5. Elesclomol changes its conformation upon chelation with copper



Nonplanar conformation of elesclomol predicted by energy-minimization

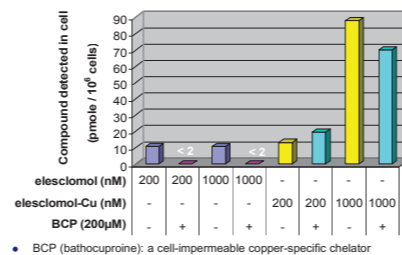
Flat-planar conformation of 1:1 elesclomol-Cu chelate as confirmed by X-ray crystallography

Figure 6. Elesclomol requires copper for cancer killing activity



- Elesclomol was not active in metal-free medium
- Supplementation of copper but not other metals recovered the activity

Figure 7. Elesclomol does not enter cells unless it chelates copper



BCP (bathocuproine): a cell-impermeable copper-specific chelator

Results

Figure 8. Elesclomol is not active unless it chelates copper

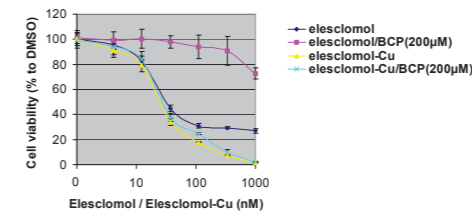
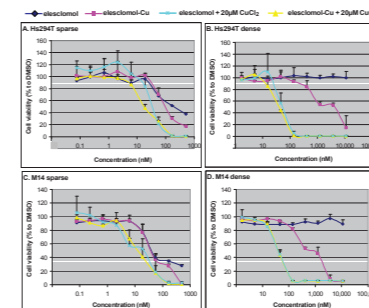


Figure 9. Elesclomol shows cell-density dependent activity, and preformed elesclomol-Cu does not



- The cancer cell killing activity of elesclomol was dramatically reduced when cells were cultured at high cell density (sparse: 2,500 cells/96-well, dense: 20,000 cells/96-well)
- 20µM of copper added to the culture restored elesclomol activity in dense cell cultures

Figure 10. Elesclomol can obtain Cu from ceruloplasmin (CP), the major Cu-carrier protein in blood

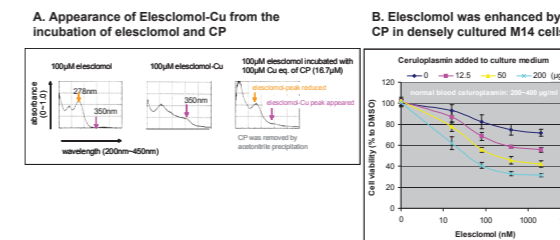


Figure 11. Elesclomol-Ni has lower redox potential than elesclomol-Cu and is inactive

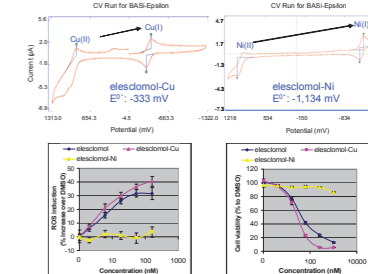
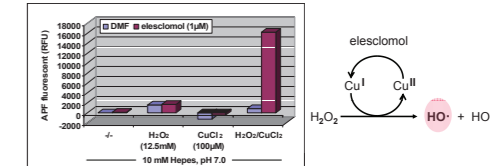


Table 1. Correlation between anticancer activity and redox potential of elesclomol structural analogues

Compound ID	redox potential (mV)	IC50 (nM)
Compound 1-Cu	6	>1250
Compound 2-Cu	-22	>1250
Compound 3-Cu	-323	62
Compound 4-Cu	-326	21
Elesclomol-Cu	-333	10
Compound 5-Cu	-338	53
Compound 6-Cu	-340	24
Compound 7-Cu	-443	>1250
Compound 8-Cu	-615	>1250
Compound 9-Cu	-778	>1250
Compound 10-Cu	-927	>1250
Elesclomol-Ni	-1,134	>1250

Figure 12. Elesclomol Generates Hydroxyl Radicals by Redox Cycling of Copper



- Hydroxyl radical was generated by elesclomol in the presence of CuCl₂ and H₂O₂

Conclusions

- Elesclomol chelates Cu outside of cells and enters cells as elesclomol-Cu(II)
- The chelation of Cu by elesclomol is required for both ROS generation and cancer cell killing activity
- 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