

#2093 Elesclomol-Cu Chelate Selectively Targets Mitochondria to Induce Oxidative Stress

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Abstract

Introduction: Elesclomol is a first-in-class investigational drug that exerts anticancer activity through elevating the level of reactive oxygen species (ROS) and oxidative stress. We recently reported that elesclomol selectively chelates Cu(II) in plasma, which causes a change in conformation that enables its uptake into cells. A cell-free assay system showed that elesclomol-Cu(II) generated ROS via the reduction of Cu(II) to Cu(I). A correlation has been observed between the redox potential and anticancer activity for Cu chelates of elesclomol and its analogs, suggesting that the ability to promote redox cycling of Cu(II) to Cu(I) is necessary for anticancer activity. Here, we demonstrate that elesclomol-Cu carries copper into mitochondria, leading to an increase in oxidative stress and apoptosis due to mitochondrial stress.

Results: The subcellular distribution of elesclomol was tracked using preformed elesclomol-Cu chelates. Cytosolic, nuclear and mitochondrial fractions of HL60 cells were prepared from cells treated with elesclomol-Cu, and total copper levels were determined for each cellular fraction. Elevated copper levels were observed only in the mitochondrial fraction, suggesting that elesclomol-Cu selectively transported copper into the mitochondria. To verify that the increased mitochondrial copper levels were from elesclomol-Cu, a copper complex of elesclomol was preformed with ⁶⁵Cu. This elesclomol-⁶⁵Cu complex was incubated for 2h with HL60 cells that were previously enriched with ⁶³Cu using ⁶³Cu-supplemented media, and subcellular distributions of ⁶³Cu and ⁶⁵Cu determined by ICP-MS. Control mitochondria contained minimal levels of endogenous ⁶³Cu. In contrast, ⁶⁵Cu was markedly increased in the mitochondrial fraction of elesclomol-⁶⁵Cu treated cells but not in the cytosolic or nuclear fractions, confirming the selective mitochondrial uptake of copper with elesclomol-Cu. Next, we compared the mitochondrial uptake of elesclomol-Cu and disulfiram(DSF)-Cu using isolated mitochondria. DSF is a Cu chelator, and Cu has been shown to enhance DSF-mediated growth inhibition and apoptosis in cancer cells through the generation of ROS. As expected, an increase in copper levels was observed in mitochondria treated with elesclomol-Cu, yet no increase in mitochondrial copper was seen following treatment with DSF-Cu at its cytotoxic concentration, emphasizing the novel mitochondrial selectivity of elesclomol-Cu. To investigate whether the mitochondrial entry of elesclomol-Cu triggered the generation of ROS, ROS in isolated mitochondria was measured. Mitochondrial ROS was immediately increased by adding elesclomol-Cu while no change in mitochondrial ROS was seen using DSF-Cu or free Cu²⁺. These results show that elesclomol induces apoptosis through elevating ROS directly in cancer cell mitochondria.

Background

Altered redox status and increased reactive oxygen species (ROS) generation are characteristic of many cancer cells. This state of elevated basal oxidative stress may represent a potential vulnerability of tumors. Disrupting this redox balance and exceeding the threshold compatible with cellular survival provides a unique strategy to selectively target cancer cells, particularly since normal cells are less sensitive to agents that induce oxidative stress due to a lower basal level of ROS production and higher antioxidant capacity.

Elesclomol has been evaluated in combination with paclitaxel in a number of clinical trials and has demonstrated therapeutic activity, including prolonged progression-free survival in advanced melanoma patients with low levels of lactate dehydrogenase (LDH) at baseline. Despite the evidence of therapeutic activity the molecular mechanisms by which elesclomol generates ROS have not been fully defined. An increased molecular understanding of the mechanism of action of elesclomol will contribute to its continued development and clinical application.

In this study we show that elesclomol generates ROS by redox cycling of Cu(II) to Cu(I) within the mitochondria and that this process is necessary for its anticancer activity. These findings provide a biological framework for understanding the unique mode of action of elesclomol. More importantly, targeting mitochondrial energy production in cancer cells represents a novel approach, entirely distinct from cytotoxic chemotherapy or kinase

Elesclomol-Cu quickly induces apoptosis in cancer cells while DSF-Cu cell killing is delayed

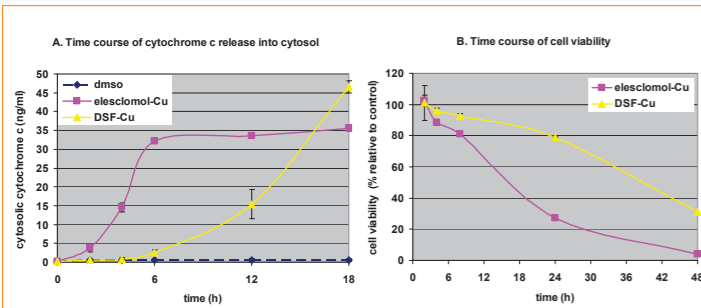


Figure 1. HL60 cells (60k cells/well-96 plate) were treated with 200nM drug-Cu complexes. A. Cytosol was fractionated. Cytochrome c was measured by ELISA. B. Cell viability was evaluated by cellular ATP level using CellTiter Glo assay

Elesclomol-Cu increases mitochondrial copper while DSF-Cu increases cytosolic copper

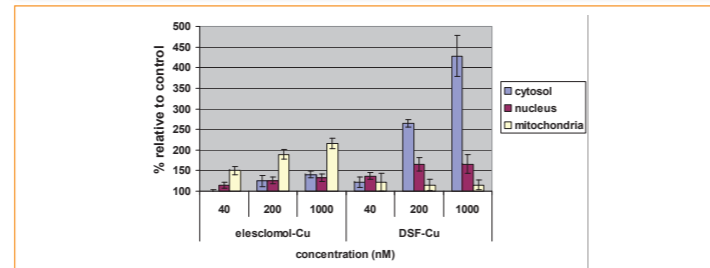


Figure 2. HL60 cells were treated with copper-complexes for 2h. Subcellular concentrations of total copper were determined by BCA assay.

Elesclomol-Cu accumulates in mitochondria rapidly and selectively

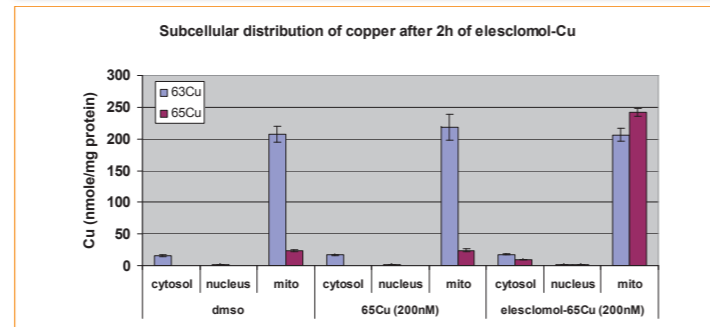


Figure 3. ⁶⁵Cu was complexed with elesclomol as a tracer. HL60 leukemia cells were fractionated after treatment with 200nM elesclomol-⁶⁵Cu for 2h. ⁶³Cu and ⁶⁵Cu were measured by ICP-MS.

Elesclomol-Cu induces rapid ROS in isolated mitochondria while DSF-Cu does not

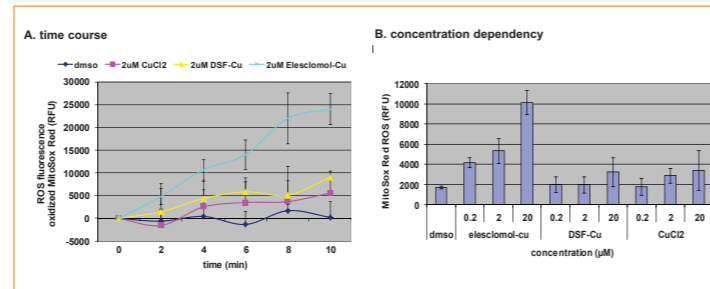


Figure 4. Mitochondria were isolated from HL60 cells. Isolated mitochondria were treated with Cu-complexes in the presence of ETC substrates. ROS production was measured with MitoSox Red.

Elesclomol-Cu(II) is reduced to Cu(I) in isolated mitochondria, but DSF-Cu(II) is not

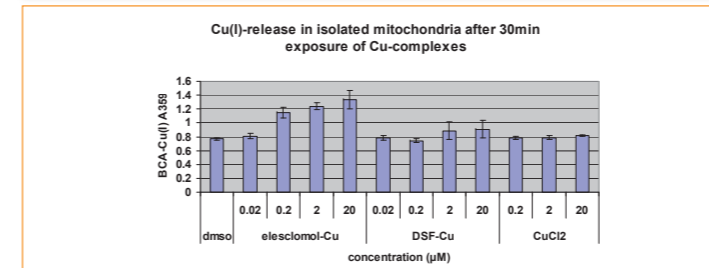


Figure 5. Mitochondria were isolated from HL60 cells. Isolated mitochondria were exposed to elesclomol-Cu(II) for 30min. Mitochondrial Cu(I) was measured by BCA assay in the absence of Cu(II)-reducing agent.

Active compounds release Cu(I) in isolated mitochondria while inactive compounds do not, suggesting Cu(I)-release dependent cytotoxicity

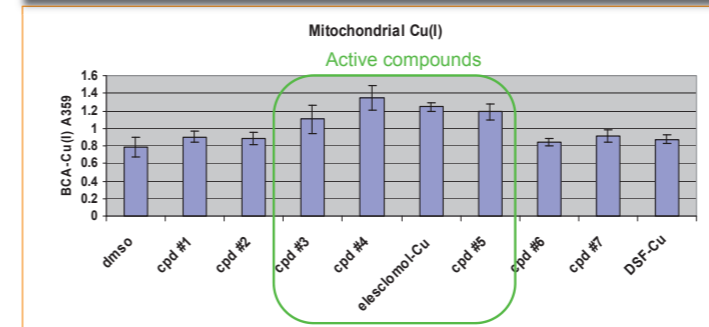


Figure 6. Mitochondria were isolated from HL60 cells. Isolated mitochondria were treated with Cu-complexes for 30min. Cu(I) was measured by BCA assay in the absence of Cu(II)-reducing agent.

Elesclomol-Cu and active analogs have a unique redox potential

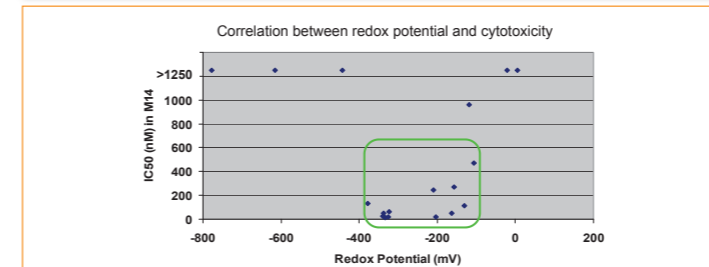
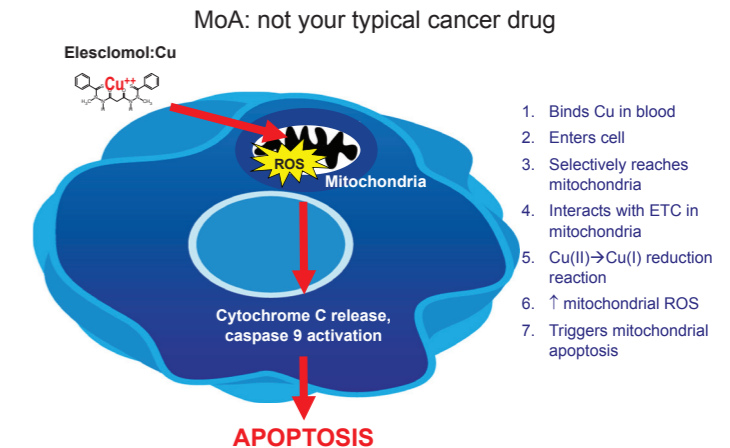


Figure 7. Elesclomol-Cu and related analogs are active when their redox potentials are between -400mV and -100mV. Strong dependence on redox potential is suggestive of an interaction with the electron transport chain and sensitivity to the mitochondria membrane potential

Summary of copper-complexes

Compound	Redox potential of Cu complex	Cu(I) in mitochondria	ROS induction in mitochondria	Cell killing*
Cpd #6	++++ (6)	- (20μM)	-	± (823nM)
Cpd #7	++++ (-22)	- (20μM)	-	± (971nM)
Cpd #5	+++ (-163)	+++	+++	+++ (83nM)
Elesclomol-Cu	++ (-333)	+++	+++ (<200nM)	+++ (144nM)
Cpd #4	++ (-338)	+++	+++	+++ (31nM)
Cpd #3	++ (-340)	+++	+++	+++ (94nM)
Cpd #2	+ (-778)	- (20μM)	-	- (>1μM)
Cpd #1	± (-927)	- (20μM)	-	- (>1μM)
Elesclomol-Ni	- (-1134)	- (20μM)	-	- (>10μM)
Disulfiram-Cu	++ (-359)	- (20μM)	- (5μM)	+ (796nM)
CuCl ₂	na	- (20μM)	- (5μM)	- (10μM)

Table 1. *HL60 cells were seeded at 25k cells/384well-plate. Cell viability was evaluated by CellTiter Glo ATP assay at 24h



Conclusions

- Elesclomol-Cu rapidly accumulates in mitochondria upon incorporation into cells
- Anti-cancer activity of elesclomol and its analogs is highly dependent on the redox potential of their copper complexes, which is suggestive of an interaction with the electron transport chain and sensitivity to the mitochondrial membrane potential
- The anti-cancer activity correlates with copper reduction in mitochondria, suggesting that the increased Cu(I) in mitochondria induces mitochondrial ROS, which in turn is capable of triggering apoptosis
- In contrast, disulfiram-Cu (DSF-Cu), an extensively investigated anti-cancer copper complex with similar redox potential to elesclomol-Cu, does not target mitochondria, and shows slower apoptosis induction and weaker activity than elesclomol-Cu
- Elesclomol induces apoptosis through a unique means of directly targeting cancer cell mitochondria and elevating reactive oxygen species

