

Abstract

Elesclomol is a first-in-class investigational drug that exerts potent anticancer activity through the elevation of reactive oxygen species (ROS) levels and is currently under clinical evaluation as a novel anticancer therapeutic. We recently demonstrated that elesclomol preferentially binds extracellular copper (Cu) and selectively transports this metal ion to the mitochondria of tumor cells to promote mitochondrial ROS generation and subsequent apoptosis. Here we report that elesclomol-induced copper transport and apoptosis is tumor selective. Comparative analysis using human PBMCs and the promyelocytic tumor cell line HL60 demonstrated that, despite similar cellular uptake of elesclomol by both cell types, copper levels increased only in HL60 cells. Subcellular fractionation showed that copper was enriched in the mitochondrial fraction of HL60 cells, but not in donor PBMCs. Further, elesclomol-Cu induces ROS in HL60-derived mitochondria but not in those isolated from PBMCs. These results suggest that elesclomol-Cu selectively targets cancer cell mitochondria to ultimately produce critical elevations in oxidative stress. *In vitro* copper accumulation was found to result from elesclomol-copper complex shuttling and was directly related to exposure time. These data predict that sustained administration of elesclomol would provide improved anticancer activity. To evaluate continuous elesclomol-Cu exposure *in vivo*, elesclomol was administered to mice using an Alzet pump at a clinically relevant dose. Using this approach, stable levels of elesclomol-Cu are achieved in contrast to the transient exposure following bolus injection. Even with this continuous exposure to elesclomol-Cu, no signs of toxicity were observed. In five different tumor xenograft models tested, elesclomol consistently demonstrated marked single agent activity with significant tumor growth suppression, indicating that continuous exposure to elesclomol-Cu results in selective and enhanced antitumor efficacy. These findings highlight a unique mechanism of action of elesclomol and support potential single agent activity of this compound in a variety of tumor types.

Background

Elesclomol exerts potent anticancer activity through the elevation of reactive oxygen species (ROS) levels, exceeding the threshold compatible with cellular survival. Recently we have shown that elesclomol readily forms a copper (Cu) chelate at a 1:1 molar ratio, resulting in a conformational change and higher hydrophobicity, and that formation of this bioactive elesclomol-Cu complex is a rate-limiting step for its activity.

Copper itself is an essential trace element for cell survival because it functions as a cofactor for several metalloenzymes and proteins involved in energy metabolism, respiration and DNA synthesis, yet is toxic when it is present at high concentrations. Copper is an excellent catalyst of redox cycling and may play a direct role in the induction of oxidative stress as a consequence of donation or acceptance of electrons inside mitochondria.

Mitochondria are indispensable for energy production, and are also crucial regulators of apoptosis. In order to control the activation of apoptotic effector mechanisms, cancer cell mitochondria are structurally and functionally altered and differ from those of normal cells. Altered redox status and increased reactive oxygen species (ROS) generation are also commonly observed in cancer cell mitochondria and an elevated basal oxidative stress represents a potential vulnerability for tumors. This biochemical property of tumor cells can be exploited for therapeutic benefit by pharmacologic ROS insults, such as that induced by elesclomol treatment. Importantly, the unique mechanism of action exhibited by the elesclomol-Cu complex represents a distinctive approach from that of chemotherapy or kinase inhibition for therapeutic intervention in human malignancies.

Copper Transport and Mitochondrial ROS Induction is Tumor Selective

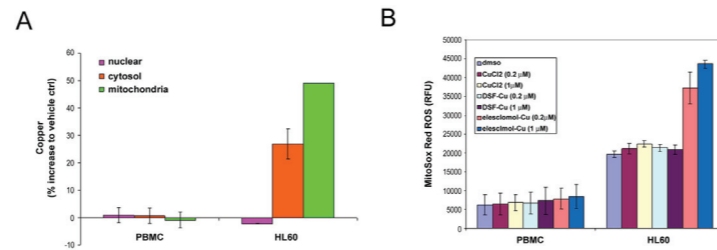


Figure 1. (A) Human PBMCs from 3 independent donors and HL-60 cells were treated with elesclomol-Cu for 2 h. Cells were fractionated into cytosolic, nuclear or mitochondrial fractions and the subcellular copper content determined by BCA assay. (B) Isolated mitochondria were treated with the indicated compounds for 30 min, and ROS levels were measured by MitoSox Red. Results are shown as mean ± SD of experiments with PBMCs from three independent donors or with three individual experiments with HL60. These data suggest that selective increase in total ROS levels to beyond a lethal threshold in cancer cells by elesclomol-Cu is not only due to the differences in basal ROS levels but also to the induction of mitochondrial ROS.

Copper Accumulation is a Result of Elesclomol-Copper Complex Shuttling Over Time

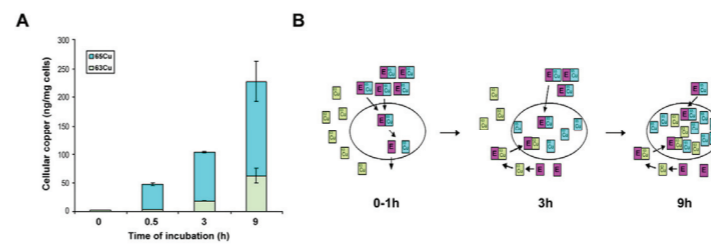


Figure 3. (A) ⁶⁵Cu was complexed with elesclomol as a tracer. ⁶⁵Cu-enriched HL-60 cells were treated with 100 nM elesclomol-⁶⁵Cu or free ⁶⁵Cu for 0h, 0.5, 3, and 9 h in 10% FBS medium, and cellular ⁶⁵Cu and ⁶⁵Cu levels were measured by ICP-MS. (B) Schema depicting elesclomol-copper shuttling and accumulation.

- These data show that the *in vitro* accumulation of copper is relative to the exposure time to elesclomol.
- This suggests a potential benefit for continuous elesclomol-Cu exposure *in vivo*.

High Cmax with Rapid Elimination by Bolus Injection vs. Sustained Elesclomol-Cu Levels by Pump

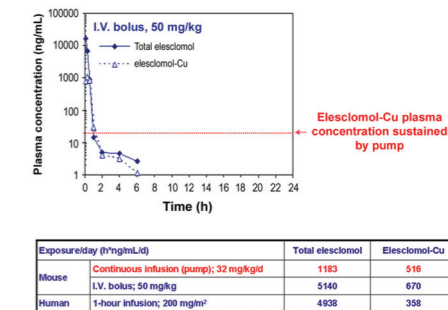


Figure 5. Elesclomol-Cu PK profiles in mice. Elesclomol salt was filled in Alzet pumps (1 μl/h) at 40 mg/ml (32 mg/kg/day, based on average body weight of 30 g) and delivered continuously for 7 days, and exposure was calculated from plasma level collected at 70 h post pump implantation.

Single Agent Activity of Elesclomol Demonstrated in a Panel of Xenograft Models

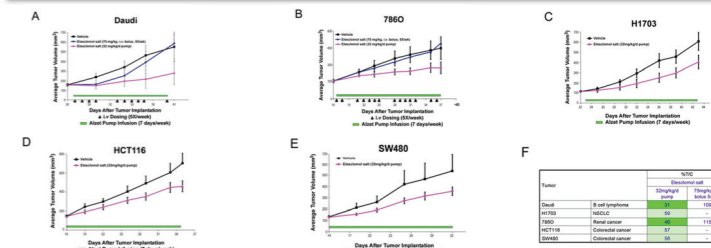


Figure 6. Tumor growth inhibition in tumor xenografts. Nude mice bearing established Daudi B cell lymphoma (A) or 7860 renal carcinoma (B) xenografts were i.v. dosed by bolus injection with elesclomol salt at 75 mg/kg 5X/week (arrowheads), or by continuous infusion of elesclomol salt at 32 mg/kg/day via Alzet pump (green bar). Points are the average tumor volume (mm³) of 6 mice. (C-E) Mice bearing established H1703 NSCLC or HCT116 and SW480 colorectal cancer xenografts were administered elesclomol salt by continuous infusion as above. Points are the average tumor volume (mm³) of 6-8 mice. (F) Summary table showing the tumor growth inhibition values (expressed as the % T/C value) in each of the xenograft models shown.

Tumor Selective Copper Transport is Not Due to Differential Cellular Uptake of Elesclomol

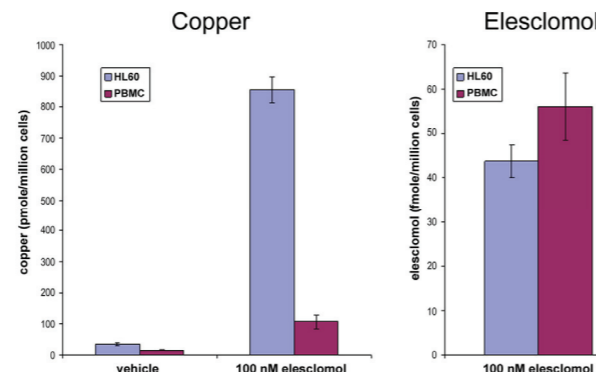


Figure 2. PBMC and HL60 cells were incubated with 100nM elesclomol and cellular copper and elesclomol levels were analyzed after a 3 h exposure. Y axis units are in piko and femto mole, respectively.

No Treatment-Related Adverse Effects Observed with Elesclomol Salt Continuously Administered via Alzet Pump

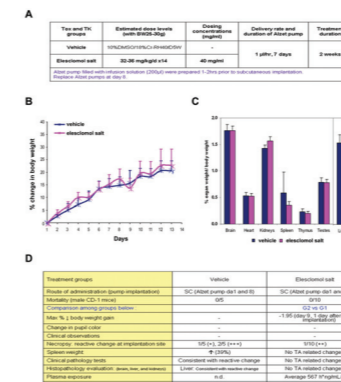


Figure 4. (A) Experimental protocol for the toxicity study assessing effects of continuous administration of elesclomol salt using an Alzet pump for two weeks. (B) No differences in body weight were observed between vehicle-treated or continuously-infused animals. (C) No treatment related differences in organ weights were seen following continuous administration of elesclomol salt. A minor increase in spleen weight associated with reactive changes at the implantation site was observed in vehicle treated animals. (D) Summary table showing the absence of any treatment-related adverse effects in mice following continuous administration of elesclomol salt via Alzet pump.

Conclusions

- Elesclomol-copper uptake is similar in normal and cancer cells, however copper accumulation and ROS induction are tumor selective.
- Copper accumulation is a result of elesclomol-copper complex shuttling over time.
- Continuous elesclomol infusion results in sustained levels of elesclomol-Cu compared to bolus administration.
- Continuous elesclomol infusion results in enhanced single-agent antitumor efficacy, with no evidence of acute toxicity.

