

Anticancer Activity of Elesclomol Correlates with Low LDH Levels and Active Mitochondrial Respiration

Masazumi Nagai, Ronald K. Blackman, Patricia E. Rao, Yumiko Wada and Keizo Koya
 Synta Pharmaceuticals Corp., Lexington, Massachusetts USA

Abstract #4545

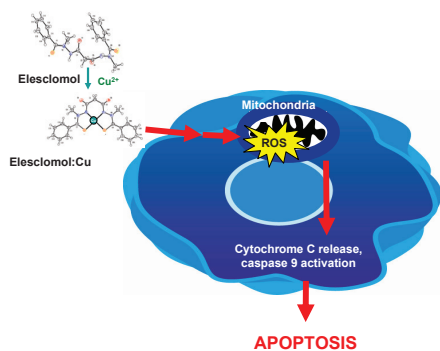
Background: Elesclomol is a first-in-class investigational drug that induces apoptosis in cancer cells through the elevation of reactive oxygen species (ROS). In a Phase 3 trial in metastatic melanoma, the level of baseline lactate dehydrogenase (LDH) in patients emerged as an important prognostic factor for treatment outcomes with elesclomol. Our previous studies revealed that elesclomol selectively chelates copper and generates ROS via reduction of Cu(II) to Cu(I). Because this copper redox reaction can be influenced by changes in cellular metabolic properties, we investigated whether elesclomol activity is influenced by the balance of mitochondrial respiration (normoxic conditions, normal LDH activity) and glycolysis (hypoxic conditions, high LDH activity) in the cancer cell. In the studies presented herein, we evaluated elesclomol activity under several situations in which cells express high levels of LDH and mitochondrial respiration is reduced.

Results: First, an increase in the level of Hypoxia Inducible Factor-1 α (HIF1 α) protein, a transcription factor that induces expression of glycolytic enzymes including LDH, was observed in M14 melanoma cells at low oxygen level or high cell density. Elesclomol showed decreased cytotoxicity under both of these conditions. Second, we evaluated lines within the same cancer phenotype but showing distinct levels of HIF1 α , and found that the high-HIF1 α -expressing Caki-2 renal cancer line was resistant to elesclomol, while lower-HIF1 α -expressing renal cancer cell lines were sensitive to elesclomol. Third, we assessed HIF1 α levels and elesclomol activity in cells treated with CoCl₂, a chemical mimetic of hypoxia. High levels of HIF1 α induction were present in M14 cells treated with CoCl₂, and elesclomol was significantly less active in these cells. The addition of oxamate, which selectively inhibits LDH-A and activates pyruvate entry into mitochondria, restored the activity of elesclomol in the CoCl₂-treated cells. These results support the hypothesis that elesclomol is more active under normoxic conditions (normal LDH) and less active under hypoxic conditions (high LDH), consistent with the clinical findings.

Introduction

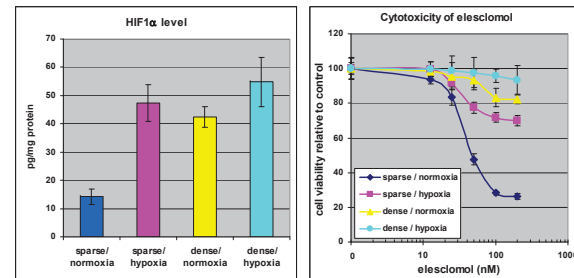
A Phase 3 trial for elesclomol in combination with paclitaxel in metastatic melanoma was conducted following a positive Phase 2b trial in the same patient population. Results were presented at ASCO in May 2009 and Perspectives in Melanoma XIII in October 2009. These results showed a differential response to treatment with elesclomol based on the level of baseline lactate dehydrogenase (LDH) in the plasma, an established prognostic biomarker in melanoma and a pre-specified stratification variable in the trial. The primary endpoint of progression-free survival was achieved in the normal LDH population, 68% of patients, with a significant improvement in median PFS (3.6 vs. 2.1 months, HR=0.76, p=0.027), an acceptable safety profile, and no impact on overall survival. In the elevated LDH population, 32% of patients, no difference was observed between the two arms of the trial for the primary endpoint (1.8 vs. 1.9 months, HR=1.10, p=0.549), and a negative impact was observed for the survival endpoint.

Results presented at the NCI-AACR-EORTC meeting in November 2009 demonstrated that elesclomol binds copper in plasma, facilitating its uptake into cells and enabling a transition between copper oxidation states. Additional research suggests that this reaction disrupts the metabolic properties of cancer cell mitochondria and generates the oxidative stress that triggers programmed cell death.



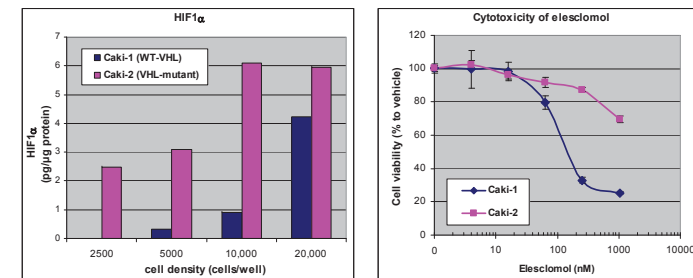
Results

Figure 1. Elesclomol is less potent in melanoma cells when HIF1 α is high



Total HIF1 α (cytosolic + nuclear) and cell viability of M14 melanoma cells were evaluated at 12h and 48h by ELISA (R&D systems) and cellular ATP levels (CellTiter Glo-Promega), respectively, under normoxia (20% O₂) or hypoxia (0.1% O₂) with sparse (2k cells/well) or dense (20k cells/well) in 96-well plates.

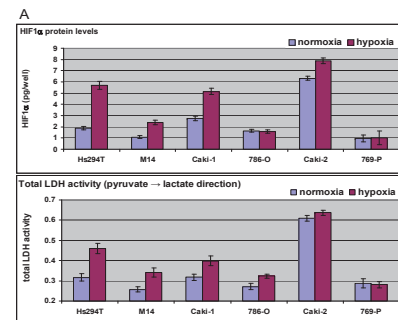
Figure 2. HIF1 α -high Caki-2 cells are resistant to elesclomol, while HIF1 α -low Caki-1 cells are sensitive to elesclomol.



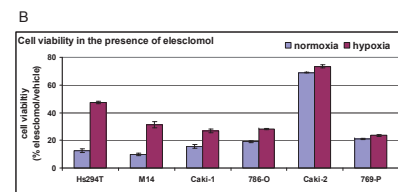
Left: Cells were serially diluted to the densities indicated. Cell lysates were harvested for ELISA at 24h after seeding. **Right:** Cells were seeded at 2,500 cells/well-96 plate. Cell viability was evaluated by CellTiter Glo ATP assay at 24h.

- Caki-1 and -2 are renal cancer cell lines established from the same patient.
- VHL protein is a negative regulator of HIF1 α .
- VHL mutation results in constitutive HIF1 α expression
- Caki-2 cells constitutively express HIF1 α while the expression in Caki-1 cells is conditional

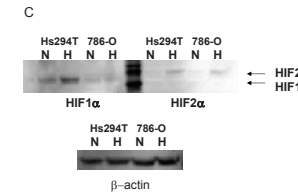
Figure 3. High HIF1 α protein and LDH-A activity levels correlate with reduced sensitivity to elesclomol



Reductive (pyruvate to lactate) LDH activity and HIF1 α level: cells were seeded at 2k cells/well in a 96-well plate and cultured under normoxia (20% O₂) and hypoxia (0.1% O₂). LDH-A activity (measuring reduction of pyruvate to lactate by monitoring NADH at 340 nm) and HIF1 α were analyzed at 24h.



Cells were treated with vehicle or 50nM elesclomol under normoxia (20% O₂) and hypoxia (0.1% O₂) for 36h. Cell viability was evaluated by cellular ATP (CellTiter Glo, Promega).



HIF1 α and HIF2 α induced by hypoxia: immunoblots were carried out with mouse monoclonal anti-HIF1 α (R&D System) and rabbit anti-HIF2 α (Abcam) antibodies. N: normoxia (20% O₂); H: hypoxia (0.1% O₂)

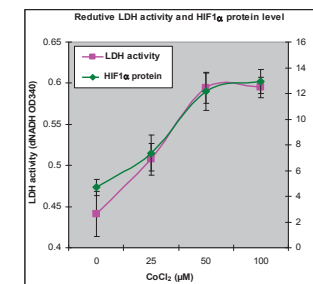
Summary of correlation of HIF1 α and LDH-A with activity of elesclomol

Cell line	Tissue	VHL*	HIF1 α		LDH-A		Viability w/ elesclomol	
			Normoxia	Hypoxia	Normoxia	Hypoxia	Normoxia	Hypoxia
Hs294T	melanoma	WT	-	-	-	-	+	+
M14	melanoma	WT	-	-	-	-	+	+
Caki-1	renal cancer	WT	-	-	-	-	+	+
786-O	renal cancer	null	-	-	-	-	+	+
Caki-2	renal cancer	mutant	++	++	++	++	+	+
769-P	renal cancer	WT	-	-	-	-	+	+

*negative regulator of HIF1 α

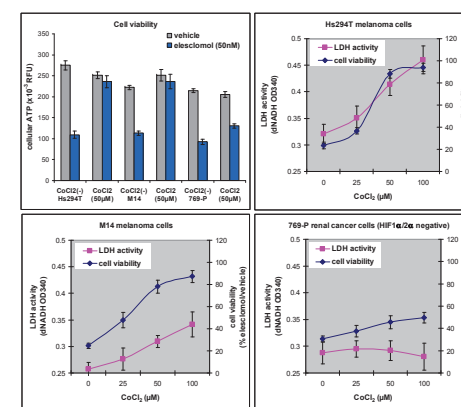
LDH-A activity increased in most of the cell lines under hypoxic conditions (786-O cells increased LDH-A activity under hypoxic conditions with an increase in HIF2 α , another HIF that increases LDH-A). 769-P cell line, which did not elevate LDH-A activity under hypoxic conditions, showed no change in its sensitivity to elesclomol.

Figure 4. CoCl₂, a chemical mimetic of hypoxia, increased HIF1 α by stabilizing HIF1 α protein resulting in increased reductive LDH activity



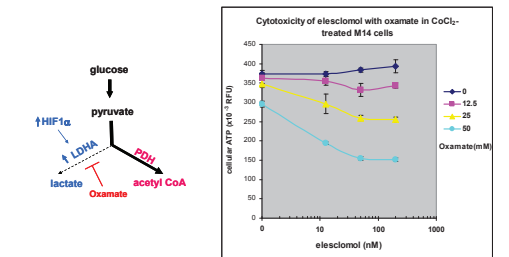
LDH-A activity (measuring reduction of pyruvate to lactate by monitoring NADH at 340 nm) and HIF1 α were analyzed after 24h incubation of M14 melanoma cells with the indicated concentration of CoCl₂.

Figure 5. Increase in LDH-A activity results in a reduction in the sensitivity to elesclomol



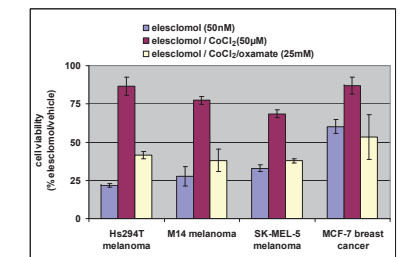
LDH-A activity was assessed after 24h incubation with the indicated concentration of CoCl₂. Cell viability with or without 50 nM elesclomol in the presence of CoCl₂ was evaluated at 48h.

Figure 6. Oxamate, a pyruvate analog and LDH-A inhibitor, restores the activity of elesclomol in CoCl₂-treated M14 cells



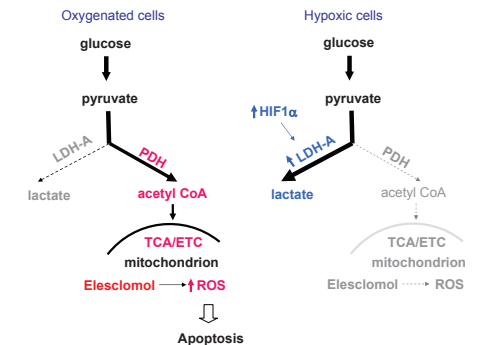
Cell viability was assessed after 48h incubation with or without elesclomol and oxamate (competitive inhibitor of LDH-A) in the presence of 50 μ M CoCl₂.

Figure 7. Elesclomol remains active in CoCl₂-treated cells when co-treated with oxamate



Cell viability was assessed after 48h incubation with or without 50nM elesclomol in the presence or absence of 50 μ M CoCl₂ and 25mM oxamate

Figure 8. Mitochondrial activity dependent toxicity of elesclomol



Conclusions

- A clear correlation was observed between the activity of elesclomol and LDH-A levels
- Elesclomol's activity was restored in LDH-A-high cancer cells by an LDH-A inhibitor, oxamate
- These results suggest that elesclomol is more active in cells where energy production is primarily through mitochondrial respiration (normoxic conditions; normal LDH-A) and less active in cells where energy production occurs primarily through glycolysis (hypoxic conditions; high LDH-A)
- These observations are consistent with results from the Phase 3 SYMMETRY trial in which elesclomol showed anti-cancer activity in the normal LDH patient population but not in the elevated LDH population
- These findings may be important for identifying patient populations for future clinical development of elesclomol

