Elesclomol induces cancer cell apoptosis through oxidative stress

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Abstract
Elesclomol (formerly STA-4783) is a novel small molecule undergoing clinical evaluation in a pivotal phase III melanoma trial (SYMMEtRY). In a phase II randomized, double-blinded, controlled, multi-center trial in 81 patients with stage IV metastatic melanoma, treatment with elesclomol plus paclitaxel showed a statistically significant doubling of progression-free survival time compared with treatment with paclitaxel alone. Although elesclomol displays significant therapeutic activity in the clinic, the mechanism underlying its anticancer activity has not been defined previously. Here, we show that elesclomol induces apoptosis in cancer cells through the induction of oxidative stress. Treatment of cancer cells in vitro with elesclomol resulted in the rapid generation of reactive oxygen species (ROS) and the induction of a transcriptional gene profile characteristic of an oxidative stress response. Inhibition of oxidative stress by the antioxidant N-acetylcysteine blocked the induction of gene transcription by elesclomol. In addition, N-acetylcysteine blocked drug-induced apoptosis, indicating that ROS generation is the primary mechanism responsible for the proapoptotic activity of elesclomol. Excessive ROS production and elevated levels of oxidative stress are critical biochemical alterations that contribute to cancer cell growth. Thus, the induction of oxidative stress by elesclomol exploits this unique characteristic of cancer cells by increasing ROS levels beyond a threshold that triggers cell death. [Mol Cancer Ther 2008;7(8):2319–27]

Introduction
Elesclomol [N-malonyl-bis (N’-methyl-N’-thiobenzoyl hydrazide); Fig. 1] is a novel anticancer agent derived from a phenotypic screen for small molecules with potent pro-apoptotic activity. Elesclomol showed antitumor activity against a broad range of cancer cell types and substantially enhanced the efficacy of chemotherapeutic agents such as paclitaxel in human tumor xenograft models (1). In a phase I clinical trial in combination with paclitaxel in patients with refractory solid tumors, elesclomol was well tolerated with a toxicity profile similar to that observed with single-agent paclitaxel (2).

In a recent 21-center, double-blinded, randomized, controlled phase II clinical trial in 81 patients with stage IV metastatic melanoma, elesclomol in combination with paclitaxel doubled median progression-free survival compared with paclitaxel alone (P = 0.035).1 Cutaneous melanoma is a highly malignant tumor derived from melanocytes, the pigment-producing cells in the epidermis of the skin. If diagnosed and surgically removed while localized in the outermost skin layer, melanoma is potentially curable. However, for patients with deeper lesions or metastatic disease, the prognosis is poor, with an expected median survival of only 6 to 9 months for patients with stage IV metastatic melanoma (3). Despite intensive efforts to improve disease prognosis, little progress has been made. Elesclomol has now entered a pivotal, confirmatory phase III clinical trial in metastatic melanoma. Additional phase II trials in other indications, as well as in combination with other agents, are planned. Despite promising clinical results, the underlying mechanism responsible for the efficacy of elesclomol has not been defined previously. Understanding the mechanism of action of elesclomol is therefore expected to yield novel pathways that can be therapeutically exploited for the treatment of melanoma and possibly other cancers.

Modulating reactive oxygen species (ROS) levels has been proposed as a therapeutic strategy to selectively target the destruction of cancer cells (4). ROS is a collective term used to describe chemical species that are produced as byproducts of normal oxygen metabolism and include the superoxide anion, hydrogen peroxide, and the hydroxyl radical (5). In normal cells, ROS are produced at low concentrations and therefore effectively neutralized by the potent antioxidant system of the cells. In contrast, cancer cells produce elevated levels of ROS due to increased metabolic activity, resulting in a state of chronic oxidative stress (6). Elevated levels of ROS promote carcinogenesis and cancer progression by amplifying genomic instability

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1 O’Day et al., in preparation.
through DNA damage and also by directly stimulating tumor-promoting signal pathways through redox modulation of key regulatory proteins (7). Moreover, ROS alone have transforming capacity because overexpression of the superoxide-generating oxidase NOX1 is sufficient to transform NIH3T3 cells (8). Thus, oxidative stress confers a growth advantage to cancer cells by activating signaling pathways that stimulate proliferation and maintenance of the transformed phenotype. In contrast to the tumor-promoting activities of ROS, excessive levels of ROS beyond the antioxidant capacity of the cells can readily induce cell cycle arrest and apoptosis. Recent studies showed the utility of agents that increase cellular ROS levels to effectively kill cancer cells (9, 10). Indeed, oncogenic transformation results in abnormal increases in ROS that render cancer cells sensitive to oxidative stress inducers (11). These inducers further elevate ROS levels beyond a threshold, ultimately triggering apoptosis. In contrast, normal cells are less sensitive to agents that induce oxidative stress due to their low level of ROS production and high antioxidant capacity. Taken together, these findings suggest that oxidative stress in cancer cells holds the potential to be exploited as novel and selective anticancer therapies.

We report here that the mechanism underlying the proapoptotic activity of elesclomol is through the induction of ROS and oxidative stress. Elesclomol rapidly induced

![Chemical structure of elesclomol.](image)

Figure 1.

Table 1. Transcripts induced by elesclomol in Hs294T cells by elesclomol

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<tr>
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<th>Gene symbol</th>
<th>Gene title</th>
<th>Fold change</th>
<th>Functional category</th>
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<td>Heat shock 70 kDa</td>
<td>421.3</td>
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<td>BCL2-associated antigen</td>
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<td>Chaperone related</td>
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<tr>
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<td>Heat shock 70 kDa</td>
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<td>Chaperone</td>
</tr>
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<td>4494</td>
<td>MT1F</td>
<td>Metallothionein 1F (functional)</td>
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<td>Metal binding/antioxidant</td>
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<td>Polyamine regulation</td>
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<td>Transcriptional regulation</td>
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<td>Cytokine/stress response</td>
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<td>56829</td>
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<td>Protein phosphatase 1, regulatory (inhibitor) subunit 15A</td>
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NOTE: Cells were treated with 100 nmol/L elesclomol for 6 h, and 266 transcripts were induced by elesclomol. The top 30 genes are shown. The complete data were deposited in the GEO database. The accession number is GSE11550 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE11550).


† Level of transcript relative to that observed for DMSO-treated cells.
ROS in cancer cells, generating a transcription profile characteristic of an oxidative stress response. Moreover, pretreatment of cells with antioxidants blocked all in vitro activities associated with elesclomol, including ROS production and the induction of apoptosis. These findings identify ROS induction as the primary mechanism of action of elesclomol and support the utility of oxidative stress inducers as anticancer agents.

Materials and Methods

Cell Culture and Chemicals

Human Hs294T melanoma cells, HSB2 T lymphoblast leukemia cells, and Ramos Burkitt’s lymphoma B cells were obtained from the American Type Culture Collection. Normal human keratinocyte cells were obtained from Lonza. All cells were grown at 37°C in medium recommended by the supplier in a humidified incubator containing 5% CO2. Elesclomol and STA-9090 (Synta Pharmaceuticals) were dissolved in DMSO at a concentration of 10 mmol/L and subsequently diluted in cell culture medium. The antioxidants N-acetylcysteine (NAC) and Tiron (Sigma) were dissolved in H2O.

Transcription Profiling

Hs294T cells were seeded at 30% to 40% confluence and incubated for an additional 3 h before treatment in triplicate with either DMSO or elesclomol. RNA was isolated from the cells at 6 h post-treatment using the RNeasy kit (Qiagen) and quality was confirmed by spectrophotometric examination. The One-Cycle cDNA Synthesis Kit was used to generate cDNA that was subsequently labeled using the GeneChip IVT Labeling Kit (Affymetrix). Analysis was then done in duplicate for each sample using the Affymetrix Human Genome U133Plus 2.0 gene chip array. Data from the Affymetrix Gene Chip Scanner 3000 were averaged and plotted.

Quantitative PCR

Total RNA was isolated using the QiaShredder and RNeasy kits (Qiagen), and 500 ng RNA from each sample was used for cDNA synthesis using the iScript cDNA Synthesis Kit (Bio-Rad Laboratories). cDNA mixture (1 μL) was added to the iQ SYBR Green Supermix (Bio-Rad Laboratories), and quantitative PCR (QPCR) was done in an iCycler (Bio-Rad Laboratories) using standard protocols. QPCR for each sample and time point was done in duplicate. The following primers were used to monitor the amounts of inducible human Hsp70 and glyceraldehyde-3-phosphate dehydrogenase transcripts: Hsp70 forward AAGGACATCGCCAGAACAGCG and reverse AAGAACTCTGCGACGCTTCG; glyceraldehyde-3-phosphate dehydrogenase forward AAGTCTCGAGTCACCGATGG and reverse CATGTTCAACCCATGGCAACA. Ct values were obtained from the iCycler program and the amount of Hsp70 RNA normalized to the glyceraldehyde-3-phosphate dehydrogenase reference was determined using the formula 2–ΔΔCT. Duplicate wells were averaged and plotted.

Apoptosis Assay

Cell death was determined by flow cytometry of cells double stained with Annexin V/FITC and propidium iodide (PI) using a Vybrant Apoptosis assay kit (Invitrogen). Flow cytometry was done using a FACSCalibur equipped with CellQuest Pro software.

Mitochondrial Membrane Potential Assay

Mitochondrial membrane potential was measured by flow cytometry using the lipophilic cation 5,5,6,6-tetrachloro-1,1,3,3-tetraethylbenzimidazol-carbocyanine (JC-1; Invitrogen). The method is based on the ability of this fluorescent probe to enter selectively into the mitochondria and on the fact that it reversibly changes its color from green to red as the membrane potential increases (over values of ~80-100 mV). This property is due to the reversible formation of JC-1 aggregates on membrane polarization that causes a shift in the emitted light from 530 nm (emission of JC-1 monomeric form) to 590 nm (emission of JC-1 aggregate) when excited at 490 nm. The ratio of red fluorescence divided by green fluorescence was determined. The ratio of red to green fluorescence was decreased in cells undergoing apoptosis and in dead cells compared with healthy cells. Following elesclomol treatment, cells were resuspended in HBSS/ Ca/Mg buffer containing the JC-1 probe (2 μmol/L) and incubated for 10 min at 37°C. Flow cytometry was done using a FACSCalibur equipped with CellQuest Pro software.

Immunoblotting

For caspase-3 analysis, whole-cell lysates were isolated using 1× cell lysis buffer (Cell Signaling) plus protease inhibitors and analyzed by immunoblotting.

References

2 http://www.ncbi.nlm.nih.gov/geo
inhibitors (Calbiochem) and immunoblotted using standard procedures. The blots were then probed with antibodies that recognize either full-length or cleaved caspase-3 (Cell Signaling). To determine the levels of cytochrome c in the cytoplasm and mitochondria, cells were fractionated and the immunoblot was probed with an antibody that recognizes cytochrome c (BD PharMingen; ref. 12). All immunoblots were probed with secondary antibodies conjugated to horseradish peroxidase and visualized using Lumiglo peroxide reagents (Cell Signaling). Images were captured using the Kodak Image Station 440.

Results

**Elesclomol Induces a Signature Transcription Profile Indicative of Oxidative Stress**

We did an Affymetrix GeneChip analysis to determine the effects of elesclomol on gene expression. Because elesclomol showed clinical activity against metastatic melanoma, we used human Hs294T melanoma cells. Cells were treated with elesclomol for 6 h and the expression of 47,000 transcripts that encompass the entire genome was monitored. Elesclomol induced the expression of 266 transcripts with a fold-induction of ≥1.5 (relative to DMSO) and a hybridization signal intensity of >500 units (Table 1). We initially focused our analysis on the top 30 genes most robustly induced by drug treatment to determine if the signature transcription profile of elesclomol could be attributed to the activation of specific signaling pathways (Table 1). Elesclomol induced the expression of many genes known to be regulated by the heat shock stress response including heat shock 70 kDa (HSPA6), heat shock 70 kDa (HSPA1B), DNAJ (Hsp40) homologue member 1 (DNAJB1), BCL2-associated anthogene (BAG3), heat shock 70 kDa (HSPA1A), spermidine (SAT), heat shock 105/110 kDa (HSPH1), crystalline αβ (CRYAB), interleukin 8 (IL-8), and DNAJ (Hsp40) homologue member 9 (DNAJB9). Most proteins encoded by heat shock responsive genes function to prevent misfolding of proteins and act as chaperones to accelerate protein refolding and renaturation (13). A second class of genes robustly induced by elesclomol was the metallothioneins, a family of metal-binding proteins with antioxidant activity (14). The metallothionein genes MT1F, MT1X, MTF1H, MTF1G, MTF1E, and MTF1M were all robustly induced by elesclomol. Although diverse forms of stress can induce either heat shock responsive genes or metallothionein genes, the induction of both heat shock and metallothionein genes together is a signature transcription profile of cells under oxidative stress (15). Both gene families are regulated by transcription factors that act as redox sensors for oxidative stress in cells (Fig. 2A). Specifically, the oxidation of select thiol groups to generate disulfide bonds within heat shock factor 1 induces trimerization and translocation of the transcription factor to the nucleus, where it binds to heat shock response elements and activates heat shock

![Figure 2. Elesclomol induces oxidative stress.](image-url)
responsive genes (16). In a similar mode of activation, oxidation of select thiol groups within metallothionein results in the release of zinc, which in turn binds to and activatesthemetal-activatedtranscriptionfactorMTF1 (17). Zinc-boundMTF1translocatestothenucleusandbindsto metal-responsive elements resulting in the induction of metallothionein gene expression. We also searched the entire list of 266 transcripts induced by elesclomol for genes known to be up-regulated by Nrf2, another transcription factorthatisactivatedbyoxidativestress (18). Oxidation of thiol groupswithintheKeap1proteinresultsinthereleaseandsubsequenttranslocationofNrf2tothenucleuswhere it inducestheexpressionofcellsurvivalresponse gene by binding to ARE promoter elements (Fig. 2A). Nrf2-dependent genes found to be induced by elesclomol included those encoding antioxidant pathway proteins (thioredoxin reductase 1, 2.9×; peroxiredoxin 1, 1.5×; and ferritin light polypeptide, 1.7×) and proteosome subunits (PSMD12, 2.3×; PSMD1, 2.2×; PSMC6, 2×; PSMC1, 2×; PSMD2, 1.7×; and PSMC4, 1.7×; data not shown). Thus, elesclomol treatment induces the activation of specific redox-sensitive transcription factors resulting in a signature transcription profile indicative of oxidative stress.

**Elesclomol Rapidly Induces the Accumulation of Intracellular ROS**

To test the hypothesis that elesclomol is an inducer of oxidative stress, we first examined drug-treated cells for the accumulation of ROS. Ramos Burkitt’s lymphoma B cells have a low antioxidant capacity; thus, this cell type was selected for a detailed analysis of ROS induction by elesclomol (15). Ramos cells were treated for 24 h and the level of intracellular ROS was monitored using the DCF-DA probe, which emits a green fluorescence on oxidation. Elesclomol induced the accumulation of ROS resulting in a substantial increase in the number of fluorescent cells as detected by fluorescent microscopy (Fig. 2B). Quantification of the fluorescent signal by flow cytometry showed a 2.4-fold increase in the ROS content of elesclomol-treated cells relative to that observed in DMSO-treated cells (Fig. 2C). Increasing the intracellular levels of the antioxidant glutathione by NAC pretreatment effectively blocked the accumulation of ROS and the induction of oxidative stress (Fig. 2B and C). These data show that elesclomol induces the accumulation of ROS in cancer cells.

**Induction of Gene Transcription by Elesclomol Is Dependent on Oxidative Stress**

Next, the role of oxidative stress in the induction of gene transcription by elesclomol was examined. We first determined whether the observed increase in intracellular ROS content occurred concomitantly with the induction of gene transcription. Flow cytometry analysis using the DCF-DA probe showed that elesclomol increased cellular ROS content by 20% as early as 0.5 h post-treatment (Fig. 3A). ROS continued to accumulate in a time-dependent manner resulting in a 385% increase by 6 h. Transcription profiling analysis identified Hsp70 as the gene most potently induced by elesclomol (Table 1). We therefore monitored the induction of Hsp70 RNA by QPCR analysis to determine the relationship between the induction of gene transcription and the accumulation of ROS (Fig. 3B). Elesclomol rapidly induced Hsp70 RNA levels resulting in a 4.8-fold increase at 1 h post-treatment. Hsp70 RNA levels continued to accumulate in a time-dependent manner resulting in a 160-fold increase by 6 h. Thus, intracellular ROS content and Hsp70 RNA levels increased in a concomitant manner in elesclomol-treated cells.

We next examined whether oxidative stress was required for the induction of Hsp70 RNA. Increasing the intracellular levels of the antioxidant glutathione by NAC pretreatment effectively blocked the induction of Hsp70 RNA by
elesclomol (Fig. 4A). Moreover, pretreatment with the superoxide anion scavenger Tiron also blocked Hsp70 RNA induction, showing that multiple antioxidants with differing mechanisms of action can block elesclomol activity. To show that NAC is not a general inhibitor of the heat shock response, we also examined its ability to inhibit the induction of Hsp70 RNA by a mechanism known to be independent of oxidative stress. Inhibition of Hsp90 results in the displacement of the transcription factor heat shock factor 1 from the chaperone complex and the subsequent potent induction of Hsp70 RNA (19). STA-9090 is a synthetic small molecule that potently inhibits Hsp90 via interaction with the NH2-terminal ATP-binding domain of the chaperone protein. NAC was unable to block Hsp70 RNA induction by STA-9090, indicating that the antioxidant is solely a selective inhibitor of redox-dependent heat shock factor 1 activation (Fig. 4B). We also confirmed that elesclomol and STA-9090 induced the expression of Hsp70 RNA via distinct mechanisms that could be differentiated using a normal cell line. Normal cells have a significantly higher antioxidant capacity than cancer cell lines (6). Human keratinocytes are one normal cell line that is particularly resistant to apoptosis induction by elesclomol. Therefore, we predicted that elesclomol might be unable to increase Hsp70 RNA levels via the redox activation of heat shock factor 1 in these cells. As expected, exposure of normal human keratinocytes to elesclomol induced little or no Hsp70 RNA as detected by QPCR analysis (Fig. 4C). In contrast, STA-9090 induced Hsp70 RNA resulting in an 80-fold increase in expression levels. Thus, the redox mechanism used by elesclomol to induce Hsp70 RNA can be differentiated in normal cells from the redox-independent mechanism of STA-9090.

To assess whether oxidative stress mediates the entire gene profile induced by elesclomol, a transcription profiling experiment was done on NAC plus elesclomol-treated Hs294T melanoma cells (Supplementary Table S1). As observed previously, elesclomol treatment alone induced a transcription profile characterized by increases in the expression of the heat shock responsive and metallothionein gene families. The addition of NAC to elesclomol-treated cells potently inhibited the induction of the entire oxidative stress gene expression profile. This result suggests that oxidative stress is responsible for the induction of most if not all genes by elesclomol and highlights the central importance of oxidative stress in the mechanism of action of elesclomol.

**Oxidative Stress Mediates Apoptosis Induction by Elesclomol**

Elesclomol is a potent inducer of apoptosis in many cell lines derived from a variety of cancers. We examined the role of oxidative stress in elesclomol-induced apoptosis (Fig. 5A). HS2T lymphoblast leukemia cells display low levels of spontaneous apoptotic and necrotic cell death as detected by flow cytometry; thus, this cell type was selected to determine the role of oxidative stress in mediating apoptosis in drug-treated cells. Exposure of HS2T cells to 200 nmol/L elesclomol for 18 h increased the number of early and late apoptotic cells (3.7- and 11-fold, respectively). NAC completely blocked drug-mediated cell death, showing that oxidative stress is required for the induction of apoptosis. To better understand the relationship between...
cell death and oxidative stress, we did an analysis in Ramos cells to determine the kinetics of apoptosis induction. A decrease in membrane potential across the mitochondrial inner membrane is a sensitive marker of early mitochondrial damage during apoptosis (20). We therefore monitored changes in mitochondrial membrane potential using the JC-1 cationic fluorescent dye (Fig. 5B). The accumulation of JC-1 in mitochondria yields both a green and red fluorescence. However, a decrease in the mitochondrial inner membrane potential due to apoptosis causes a decrease in red fluorescence that can be easily monitored by flow cytometry. Elesclomol treatment of Ramos cells for 1 h resulted in little or no change in mitochondrial membrane potential. An 8% decrease was observed at 3 h and a 57% decrease in mitochondrial membrane potential was observed at 6 h. Thus, the earliest mitochondrial changes associated with apoptosis occur at 3 to 6 h in elesclomol-treated Ramos cells. Monitoring the release of cytochrome c from mitochondria and caspase-3 processing by immunoblot analysis revealed the earliest occurrence at 6 h in elesclomol-treated cancer cells (Fig. 5C). Taken together, these findings show that the induction of apoptosis follows the accumulation of ROS in cells and confirms the crucial role of oxidative stress in elesclomol-induced apoptosis.

Discussion

The presented data show that elesclomol causes apoptosis in cancer cell lines through the induction of oxidative stress. Our finding that elesclomol induced a transcription profile characteristic of oxidative stress indicates the activation of specific redox sensors and pathways within drug-treated cells. Indeed, the induction of gene families...
encoding heat shock proteins and metallothioneins is a signature transcription profile that has been observed previously for cells treated with either hydrogen peroxide (21) or redox-active compounds alone (15). Besides playing a role in buffering oxidative stress, thiol-dependent redox modulation of protein activities can also regulate cell proliferation, cell cycle progression, and apoptosis (22). Both the intensity and the duration of the oxidative stress stimulus determine the final response of the cells to the insult by regulating the kinetics and diversity of the pathways activated. The activation of gene families encoding protective and/or antioxidant proteins is indicative of an initial survival response in response to elesclomol-induced oxidative stress (Table 1). This attempt by the cell to mitigate oxidative damage is both transient and futile as redox-regulated, proapoptotic signaling pathways eventually predominate the response.

The inhibition of both gene expression and apoptotic cell death by the antioxidant NAC is strong evidence that apoptosis induced by elesclomol is mediated exclusively through oxidative stress. To rule out inhibition due to a direct chemical interaction between NAC and elesclomol, we showed that the antioxidant Tiron also blocks induction of Hsp70 RNA by elesclomol. Moreover, the amount of NAC required to block elesclomol activity corresponded to the millimolar concentration range shown by others to confer antioxidant activity (23). The ROS-dependent mechanism by which elesclomol induces apoptosis has yet to be determined. Sustained levels of excess ROS are known to induce apoptosis through multiple mechanisms and pathways. Oxidative stress directly activates p53 proapoptotic signaling (24). In addition, ROS modulates ASK-1 kinase function resulting in proapoptotic JNK and p38 activation (25). ROS can also induce apoptosis by regulating the phosphorylation and ubiquitination of Bcl-2 family members (26). Finally, ROS can initiate apoptosis by directly disrupting mitochondrial membrane potential leading to the release of cytochrome c (27).

Oxidative stress and elevated ROS levels have been associated with apoptosis induced by numerous anticancer chemotherapeutic agents (28). In the case of many conventional chemotherapies, the induction of oxidative stress may be due to increased ROS generation by mitochondria initiating early apoptosis and is secondary to the primary mechanism of action of the anticancer agents. Therefore, it is perhaps unsurprising that increased ROS levels are associated with apoptosis induced by many different chemotherapeutic agents. Our data on the kinetics of ROS generation in elesclomol-treated cells confirm that oxidative stress induction by elesclomol is very rapid and precedes the earliest events associated with apoptosis (Figs. 3A and 5A-C). This finding indicates that the induction of ROS by elesclomol is not a direct consequence of mitochondria initiating apoptosis. In contrast to the action of elesclomol, transcription profiling of cancer cells following treatment with the anticancer agent paclitaxel showed no induction of an oxidative stress response transcriptional program, suggesting that any increase in ROS following paclitaxel treatment could be subsequent to the commitment to apoptosis.

The molecular mechanism by which elesclomol generates ROS is presently not understood. Certain cytotoxic agents have also been found to generate ROS by targeting mitochondria and blocking electron transfer along the electron transport chain (29). Elesclomol does not appear to generate ROS through this mechanism because drug treatment of isolated mitochondria had no effect on electron transport. Our current hypothesis is that elesclomol may induce oxidative stress by directly stimulating ROS-generating enzymatic complexes in the cytoplasm. Studies are underway to determine the mechanism of ROS induction and the molecular target of elesclomol.

Oxidative stress has recently reemerged as an anticancer therapeutic strategy (11, 15). Previous studies suggested that cancer cells, due to their high metabolic activity, produce higher levels of ROS than do normal cells (6). More recently, elevated levels of ROS in transformed cells have been associated with the activation of select oncogenic signaling pathways (11). Interestingly, transformed cells are thought to use ROS signals to stimulate both proliferation and tumor progression. This reliance on high ROS levels renders cancer cells vulnerable to further increases in oxidative stress that force the cell beyond a threshold resulting in apoptosis. Thus, agents that either increase ROS generation or decrease the amount of antioxidants have potential to target the destruction of cancer cells with little or no effect on normal cells. We observed that elesclomol induces Hsp70 as well as cell killing in a large number of different human cancer cell lines. Our determination that both activities are dependent on oxidative stress indicates that elesclomol exploits the high levels of ROS in cancer cells to potently induce apoptosis. Induction of Hsp70 RNA and apoptosis was not observed in human keratinocytes treated with elesclomol, showing that certain normal cells are resistant to the cytotoxicity associated with oxidative stress. Thus, elesclomol may show increased activity in cancer cells due to their transformed phenotype and/or higher levels of metabolic activity.

Elesclomol is currently used in clinical trials in combination with paclitaxel. In addition to the targeted disruption of microtubules, paclitaxel treatment of cancer cells in vitro also has been associated with the induction of ROS and the activation of intracellular signaling pathways (30–32). The proapoptotic activity of paclitaxel has been found to be inversely correlated to the total antioxidant capacity of cells, indicating that oxidative stress or ROS increase the cytotoxicity of paclitaxel (31). Thus, elesclomol may increase the efficacy of paclitaxel by activating signaling pathways that combine with those activated by paclitaxel to induce a potent proapoptotic signal within the cancer cell and/or by elevating ROS leading to enhanced proapoptotic activity of paclitaxel.

Elesclomol displayed significant therapeutic activity in combination with paclitaxel against metastatic melanoma in a phase II clinical study. Our finding that elesclomol induces oxidative stress and apoptosis in cancer cell lines...
in vitro provides a possible mechanism for its clinical activity. Melanoma is a unique cancer type in that melanin biosynthesis produces high levels of ROS and oxidative stress (33, 34). As a result, melanoma cells are expected to be particularly sensitive to therapeutic strategies that induce cancer cell death through further ROS induction. Other cancer types that may be especially sensitive to the prooxidant mechanism of elesclomol include breast, ovarian, prostate, and leukemia (6, 35–39). Our finding that elesclomol is an inducer of ROS highlights the potential of oxidative stress inducers as novel therapeutics for the treatment of metastatic melanoma and other cancers.

Disclosure of Potential Conflicts of Interest
All authors are current or former employees of Synta Pharmaceuticals Corp. J. Barsoum, shareholder of Synta Pharmaceuticals Corp.

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