

Repositioning an Old Anti-Alcoholism Drug: Disulfiram as a Selective, Effective and Economical Anticancer Agent

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Editorial

Disulfiram (tetraethylthiuram disulfide, or DSF), is a disulfide derivative of N, N-diethyl dithiocarbamate (DEDTC) that has been used since the 19th century in the manufacturing of rubber. In the late 1930's, researchers became interested in the use of DSF as treatment for alcoholism, after it was noticed that workers exposed to the compound while manufacturing rubber suffered severe adverse effects to alcohol and began to avoid consuming alcoholic beverages. This was found to be caused by DSF's inhibition of aldehyde dehydrogenase (ALDH), an enzyme essential to alcohol metabolism [1]. Today, DSF is an FDA-approved drug for the treatment of alcoholism, and at low doses continues to be a useful treatment option for those suffering from the disease [2].

Recent studies have shown that DSF may potentially be a good drug candidate for the treatment of some cancers [1]. DSF is a member of the dithiocarbamate family, and is thus a metal chelator. Many cancer cells have elevated levels of copper, a metal to which DSF can bind. Thus, DSF is of great interest in cancer research as an antitumor agent, as studies suggest the DSF-copper complex may be cytotoxic to cancer cells. This could allow for targeting of some types of cancer cells with elevated copper levels by DSF [3,4]. Targeting copper-enriched cancer cells by DSF could be tumor-specific since normal cells *in vivo* contain much lower levels of copper and would not be affected by the copper-dependent inhibition activity of DSF. Indeed, cultured tumor cells containing high copper similar to that found *in vivo* were sensitive to DSF treatment. It has also been shown that some compounds containing copper can inhibit the 26S proteasome activity in tumor cells, leading to apoptosis induction. This makes the disulfiram-copper complex (DSF-Cu) a promising complex to study in the treatment of cancer [5].

Various mechanisms have been proposed to be responsible for the cytotoxic effect of DSF on cancer cells. In the body, DSF is converted to its metabolite, diethyl dithiocarbamate (DTC). When complexed with metals, DTC shows anticancer activity in breast, prostate, glioblastoma, lung, melanoma, cervical, and colorectal cancer as well as myeloma and leukemia. This complex is formed in order to stabilize metal ions with unusually high or low oxidation states like copper (II), iron (III), cadmium (II), and zinc (II), which are stable depending on their resonance forms and the delocalization of the positive charge from the metal atom to the periphery of the complex [6-15]. This delocalization of electrons causes a variety of different cellular responses. One of these is an altered cellular redox environment, inducing different ROS-dependent pathways such as inhibition of NF- κ B (a protein complex that regulates many genes and may prevent apoptosis in cancer cells), alterations in expression of antioxidant enzymes such as catalase and

manganese superoxide dismutase or copper/zinc superoxide dismutase, and altered localization of proteins related to intrinsic apoptotic pathways like BAX, Bcl-2, BAD and cytochrome C. Another mechanism that has been proposed is DSF's irreversible inhibition of aldehyde dehydrogenase (ALDH), a NAD⁺-dependent enzyme which is altered in redox environments via the formation of a stable intermolecular mixed disulfide bond between DSF and an active site thiol on ALDH. DSF can also inhibit the proteasome which is associated with inhibition of NF- κ B or activation of the MAP-ERK pathway. Finally, DSF has shown synergistic effects with some anticancer agents like auranofin, telozodamide, cis-platinum, gemcitabine, and nelfinavir [16-20].

Many studies have shown DSF to be a promising compound for cancer treatment. One such study examined disulfiram's use against breast cancer cells when complexed with copper. Since cells from many different types of cancer have been found to have abnormally high levels of copper, researchers aimed to see whether this could be used as a target when treating with DSF in an effort to inhibit the proteasome. Researchers treated two breast cancer cell lines and one normal breast cell line with DSF and found that in the two malignant lines, chymotrypsin-like proteasomal activity was inhibited. Furthermore, they found that apoptosis was induced in these malignant cells following inhibition of the proteasome. However, they found that the DSF-Cu complex had no effect on the proteasome in the normal breast cell line. There was also little to no evidence of apoptosis in normal breast cells, indicating that copper-rich malignant breast cancer cells can be selectively targeted by DSF to induce apoptosis via inhibition of the proteasome [1].

A similar study aimed to test the effects of copper-complexed DSF in prostate cancer cell lines. Since previous studies with DSF alone had showed very little effect in prostate cancer cells, the researchers administered copper alongside DSF to hormone-sensitive and castrate-resistant prostate cancer cell models and found that the combination caused significant inhibition of tumor cell growth as well as induction of apoptosis. The study also more closely investigated DSF's effect on AR-positive cell lines, as AR-positive prostate cancer patients often do not respond well to endocrine therapy. The researchers found that AR-positive cells have high levels of expression of CTR1, a copper transporter protein, as well as ATP7B and STEAP4, two other copper homeostasis proteins, and that expression levels can be increased by androgens. This suggests that AR-positive cells that have upregulated these proteins could be sensitive to DSF-Cu [21].

Another study examined DSF's effect on the phosphoinositide 3-kinase (PI3K)/PTEN/AKT signaling pathway, a survival pathway important in breast cancer progression. The study found that DSF, when complexed with copper, potently inhibited growth of breast

cancer cells. Additionally, they saw this effect in both breast cells positive and negative for PIK3CA mutation. This mutation is very common in breast cancer cell lines and results in overexpression and amplification of the gene, which in turn results in activation of the PI3K/AKT signaling pathway. The researchers found that DSF-Cu treatment caused down regulation of PTEN protein in multiple cell lines, as well as pAKT activation and cell death. Next, hypothesizing that down regulation of PTEN protein and AKT activation could make cells more dependent on the PI3K/AKT pathway; they tested a combination of DSF-Cu and LY294002, a PI3K inhibitor. The study found that the combination resulted in more effective growth suppression in multiple breast cancer cell lines, probably due to inhibition of DSF-induced, PI3K-mediated survival signaling. Researchers also found that growth suppression was greater in cell lines with the PIK3CA mutation. Like the other studies, they also found proteasome inhibition and apoptosis in cells treated with the DSF-Cu and LY294002 combination, suggesting that a PI3K inhibitor such as LY294002 and a proteasome inhibitor such as DSF-Cu may be a good combination for breast cancer therapy [22].

In addition to breast cancer and prostate cancer, DSF has also been studied in malignant pleural mesothelioma (MPM) cell lines. A recent study found that cell proliferation in several MPM lines could be significantly inhibited by treatment with DSF-Cu. The researchers also studied DSF-Cu's role in apoptosis induction in mouse MPM cell lines. By examining cellular levels of caspases 2, 3, 8, and 9, all of which are involved in apoptosis signaling, they found that DSF-Cu could stimulate caspase-3 activation in several cell lines. In another MPM cell line, they saw both caspase-3 and caspase-9 activation after DSF-Cu treatment. Next, similar to the other studies, they examined the effect of DSF-Cu on the proteasome in MPM cell lines. To do this, they measured levels of ubiquitinated proteins, since the proteasome degrades proteins tagged with polyubiquitin. They found elevated levels of ubiquitinated proteins in cells treated with DSF-Cu, indicating proteasome inhibition. Like the other studies, they also found that proteasome inhibition led to induction of apoptosis in different cell lines, suggesting that these are some of the mechanisms behind DSF-Cu's suppression of cancer cell growth in their lines. Another mechanism studied was DSF-Cu's impact on NF- κ B signaling. NF- κ B is an anti-apoptotic transcription factor and is regulated by upstream kinase inhibitors, I κ B α and I κ B β . I κ B α / β is ubiquitinated and degraded by the proteasome, allowing NF- κ B to fulfill its anti-apoptotic role. The study found that cells treated with DSF-Cu had increased levels of I κ B α and I κ B β in both human and mouse MPM lines, which suggests that DSF-Cu can block the NF- κ B signaling pathway in MPM cells [23].

Together, these studies along with many others suggest that DSF is an extremely promising drug not only for the treatment of alcoholism, but also multiple cancers when complexed with copper. Evidence of DSF's ability to inhibit the proteasome and induce apoptosis in cancer cells is rapidly accumulating, but DSF also has other advantages that make it a promising drug candidate. Perhaps most notable is the fact that DSF is a very old drug and has been in use in humans for many years now as an FDA-approved anti-alcoholism drug, so its safety and pharmacology profiles are better known than a newer drug. In addition, DSF proves to be a very safe drug in both human cancer trials and alcoholism treatment and is well-tolerated with few side effects, even with long term use [24-26].

Another advantage to using DSF as an anticancer drug is the fact that it has already undergone the drug development process. Drug development is a costly, time-consuming endeavor. Today, the average

drug costs around \$2.6 billion to bring to market and takes between 10 and 20 years from synthesis to application for FDA approval [27-29]. As DSF has already been synthesized and developed, the time and cost for drug repositioning of DSF to include cancer therapy would likely be far less than the development of a novel compound. Additionally, DSF itself is a very inexpensive drug. Its current FDA-approved form for treating alcoholism, manufactured under the trade name Antabuse[®], is an oral tablet and costs on average only \$78 per prescription as of 2007 [30]. In contrast, many cancer drugs can cost thousands of dollars per year—for example, goserelin acetate, an FDA-approved drug for the treatment of breast and prostate cancer can cost over \$5,000 for two years [31]. This makes DSF an extremely cost-effective treatment option, especially when compared to current cancer drugs.

DSF is showing great promise in research studies as an effective anticancer drug. In addition to mounting evidence of its ability to inhibit the proteasome, inhibit survival pathways, and induce apoptosis in cancer cells, DSF has several prominent advantages if used as a repositioned drug. Overall, continued research on DSF is highly recommended in the pursuit of its use as an effective, low-toxic, and highly cost-effective anticancer therapy.

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