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Disulfiram Copper (II) complex targets cancer cells through generation of reactive oxygen species

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Abstract

Disulfiram (DS), a well-known prescription for managing and treating alcoholism, may be a viable repurposed medication for improving cancer therapy, particularly for treating aggressive and treatment-resistant malignancies. By raising intracellular copper (Cu) levels, producing reactive oxygen species (ROS), and triggering apoptosis, it exhibits potent cytotoxicity against a number of malignant tumor cells, especially when paired with copper. It overcomes drug resistance, inhibits cancer stem cells, and has shown promise in preclinical models of glioblastoma, breast, cervical, and pancreatic malignancies. The results of this study demonstrated that extracellular reactive oxygen species (ROS) in the medium comprising of DS/Cu and H_2O_2 produced were greater than ROS generated in the medium comprising of DDC-Cu, DS only, and Cu only. This suggests that the rapid cell death seen in the resistant breast cancer cell-lines and the destruction of cellular structures may be caused by ROS generated by the DS plus Cu reaction.

Keywords: Reactive Oxygen Spieces; Disulfiram; Cytotoxicity; Cancer Cells; Cell Death

1. Introduction

Chemically activated oxygen molecules also known as reactive oxygen species (ROS) usually have one unmatched electron in their outmost electron shell thus they are extremely excited. Superoxide (O_2•−), hydroxyl radical (•OH), nitric oxide (NO•), and non-radical oxygen derivatives like hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) are some examples of ROS. ROS are produced during normal cellular metabolism processes, however cell growth and survival depend on maintaining the levels of ROS produced. The amount of ROS produced is strictly regulated by certain antioxidants and ROS-scavenging enzymes (Boonstra and Post, 2004).

Cancer is not a idiosyncratic disease; rather, it is a broad family of disorders characterized by uncontrolled and abnormal cell proliferation caused by DNA abnormalities that can travel to other parts of the body via metastasis. With around 10 million deaths in 2020, it is progressively turning into a worldwide pandemic (Ferlay et al., 2020). Breast, lung, colon, rectum, and postate cancer are the most often diagnosed cancers. Surgery, radiation, immunotherapy, chemotherapy, and molecular-based therapy (endocrine and biological therapy) are all used to treat cancer.

Due to enhanced peroxisome activity, mitochondrial malfunction, oncogenic activity, immune cell infiltration, and elevated cellular receptor signals, cancer cells, are metabolically excited and have heightened oxidative stress and thus exhibit raised ROS levels (Storz, 2005). Cancer cells adjust to the increase ROS by accelerating the expression of cell survival proteins, change protein structures, enhance transcription factors, and stimulate genes that modulate cell evolution and differentiation (Davies, 1999). Cancer cells also possess elevated amounts of antioxidant enzymes such

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as the superoxide dismutase (SOD), catalase, peroxidases and activation of the glutathione system (GSSG/2GSH) and thioredoxin system which uphold cellular redox equilibrium (Schafer and Buettner, 2001).

The creation of new medications to fight cancer remains a significant challenge in chemotherapy despite advances in medical research because some of these cytotoxic agents cause resistance after repeated use (Koual et al., 2020). Additionally, the process of producing new medications is costly and time-consuming (Bertolini et al., 2015), which is why there is a growing interest in repurposing already-approved medications to treat novel conditions.

Even at relatively large dosages of 300 to 500 mg per day, disulfiram (DS), a first-line anti-alcoholism medication, used for more than 6 decades have few and controllable adverse effects (Suh et al., 2006). According to a number of studies, DS exhibits strong cytotoxicity against a number of cancer cells when combined with copper (Cu) (Yip et al., 2011; Liu et al., 2013; Tawari et al., 2015). According to Boonstra and Post (2004), the DS/Cu combination fundamentally boosts the production of reactive oxygen species, which causes oxidative stress that destroys DNA, proteins, and lipids and eventually causing apotosis of cancer cells. The presence of copper (II) ions is crucial for DS's anticancer activity (Gupte and Mumper, 2009). The complex formed from DS and Cu, Cu(DDC) causes apoptosis, inhibits the proteasome–NF-κB pathway, targets ALDH-positive cancer stem-like cells, suppresses tumor proteasome activities, increases the potency of other chemotherapy drugs like paclitaxel and cisplatin, and reverses acquired resistance, particularly in resistant cell lines (Yip et al., 2011; Liu et al., 2013).

Copper an essential trace metal is vital to human biology (Harris and Gitlin, 1996) and through the Fenton's reaction, it produces ROS with an oxidizing agent that damages cellular components such proteins, lipids, and DNA and triggers apoptosis. Cu, zinc (Zn), gold (Au), and iron (Fe) are among the divalent metallic ions that DS effectively chelates (Morrison et al., 2010). Consequently, an additional increase in ROS levels following exposure to DS associated with Cu may serve as a "Achilles heel" for cancer cells, ultimately causing their demise.

1.1. Objective and aim of the study

We investigated whether the ROS produced by the DS/Cu can kill cancer cells in two resistant breast cell lines, BT $549_{GEM100nM}$ and MCF-$7_{5FU10\mu M}$, which demonstrated pan and cross-resistance to a number of traditional anticancer medications from our earlier studies (Tawari Erebi Patricia, 2024; Tawari Erebi Patricia and Ere Melvin Peremobowei, 2024).

2. Methodology

2.1. Cell lines and reagents

Parental cell lines BT 549 and MCF-7. were obtained from ATCC in Middlesex, UK. By continuously growing the parental cell lines BT 549 AND MCF-7 in a stepwise concentration increasing procedure with media containing Gemcitabine (dFdC) (Sigma, Dorset, UK) and 5-Fluorouracil (5-FU) the resistant cell lines BT $549_{GEM100nM}$ and MCF-$7_{5FU10\mu M}$ were produced (Tawari Erebi Patricia, 2024; Tawari Erebi Patricia and Ere Melvin Peremobowei, 2024). Disulfiram (DS), polymeric micelles, copper chloride (CuCl2), and 99.9% dimethylsulfoxide (DMSO) were supplied by Sigma (Dorset, UK); DMEM and fetal calf serum (FCS) were supplied by Lonza (Wokingham, UK).

2.2. Reactive oxygen species activity detection

The Fc OxyBURST dihydro-2', 4, 5, 6, 7, 7'-hexafluorofluorescein (H2HFF) probe (Invitrogen, Paisley, UK) was used to measure the extracellular ROS levels. 100 µl was aliquoted into a 96-well plate after 60 µl of Fc oxyBurst dye was added to 3 ml of serum-free medium. Three wells in the 96-well plate were filled with 100 µl of 20µM H_2O_2 prepared in PBS. DS and $CuCl_2$ were added to the growth medium at equal molar concentrations (10 µM or 10 mM). A Fluoroskan Ascent UV fluorometer (Thermo Scientific, Northumberland, UK) was used to quantify fluorescence in 96-well plates at excitation 490 nm and emission 520 nm at various time intervals.

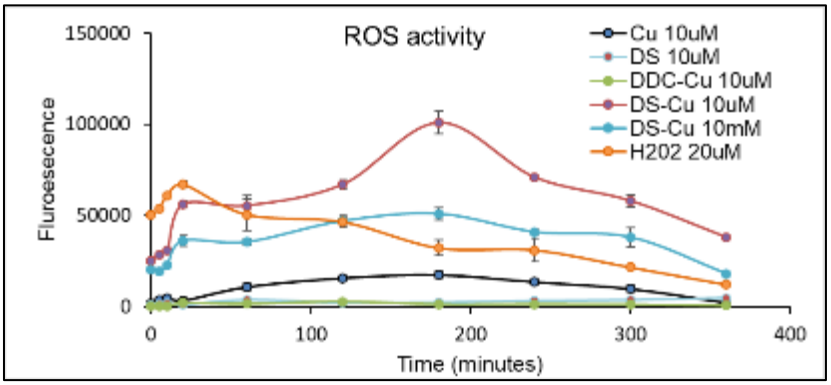
2.3. Cell culture and cytotoxicity analysis

The cell lines were grown in Dulbecco's modified Eagle's medium (DMEM) (Lonza, Wokingham, UK) supplemented with 10% FCS, 50 units ml_1 penicillin, and 50 mgml_1 streptomycin. BT $549_{GEM100nM}$ cells were preserved in a medium incorporated with 100nM of GEM, whereas MCF-$75_{FU10\mu M}$ cells were maintained in a media containing 10uM of 5-fluorouracil. Before being put through a typical MTT assay for the *in vitro* cytotoxicity test, the overnight-grown cells (5 X 10^3 per well) on 96-well flat-bottomed microtiter plates were treated with DS, Cu, and DS-Cu for 72 hours (Plumb et al, 1989).

3. Results

3.1. Generation of ros

DS/Cu, DDC-Cu, DS alone, Cu alone, and H_2O_2 reactions were used to analyze extracellular ROS generation in media. The amount of ROS in the media is indicated by the fluorescence's intensity. According to the result (Figure 1), higher amounts of ROS were found at the standard working concentration of DS at 10µM, the interaction between DS and Cu produced much more ROS than 20µM H_2O_2 and leveled after roughly three hours. Because DS and Cu crystallize instantly at greater concentrations, lower amounts of ROS were found from 10 mM of DS/Cu. No discernible ROS was noticed in DDC-Cu and DS only mediums signaling that ROS may be liable for the cytotoxicity of DS/Cu but not DDC-Cu.



(ROS: reactive oxygen species, DS: disulfiram, Cu: copper, DDC-Cu: diethyldithiocarbamic copper complex).

Figure 1 Plots of ROS activity Identified in DS, Cu, DS/Cu, DD-Cu and H_2O_2 containing cell culture medium.

3.2. Cell viability assay of free ds, cu alone and ds-cu on resistance cancer cell line (BT $549_{GEM100nM}$ and MCF-$7_{5FU10\mu M}$)

The findings of the cell viability analysis (Figure 2) demonstrated that the DS/Cu combination had a lower IC50 than free Ds or Cu alone, making it extremely cytotoxic to both resistant cell lines.

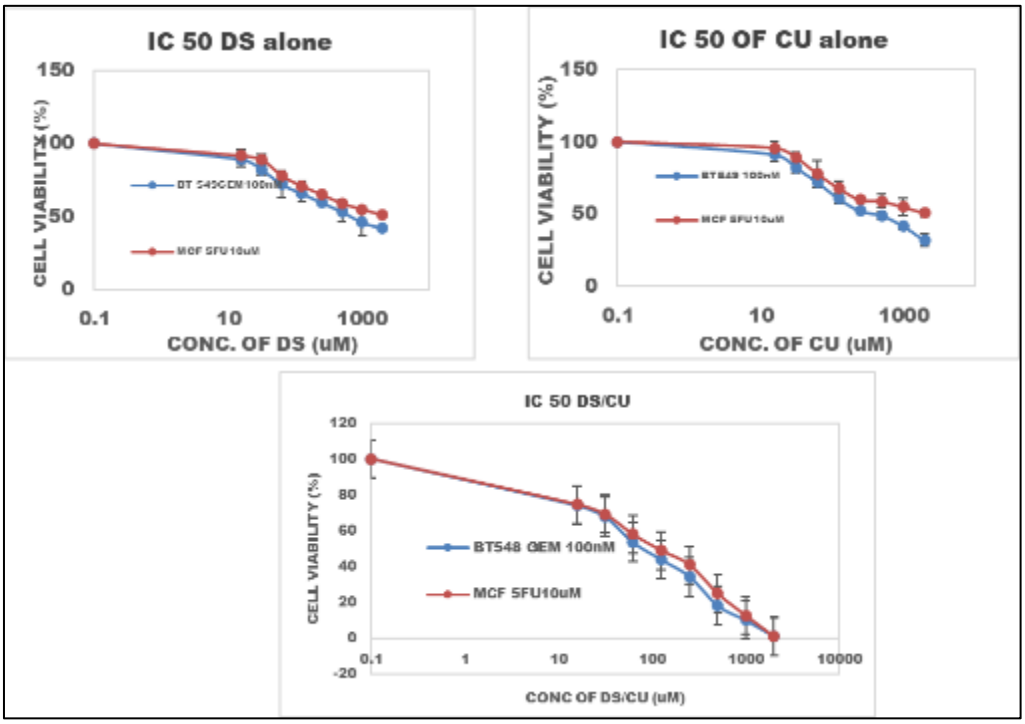


Figure 2 Drug Concentration Response Curves of BT $549_{GEM100nM}$ and MCF-$7_{5FU10\mu M}$) treated with DS alone, Cu alone and DS-Cu

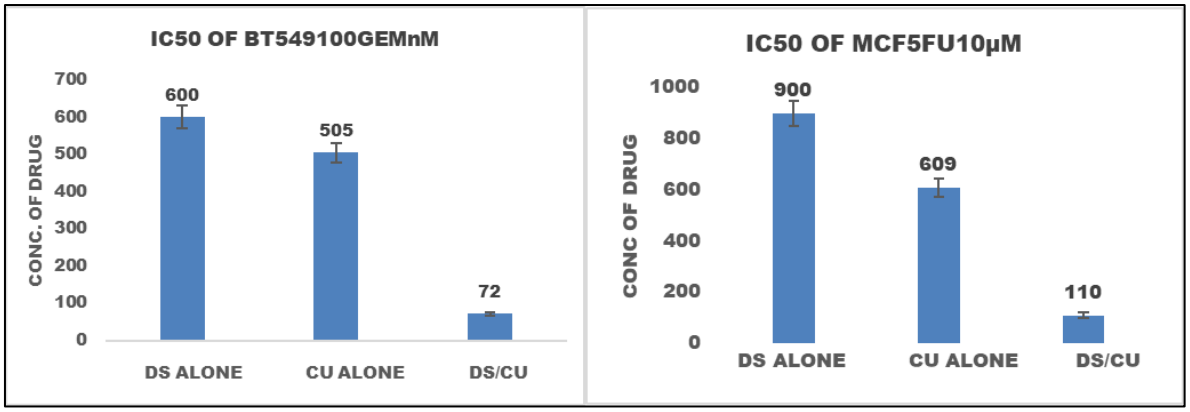


Figure 3 Histograph of Resistant cells treated with Free DS, Cu alone and DSCu

4. Discussion

Due to its strong cytotoxic effect against cancer cells, particularly in the presence of copper, disulfiram, a well-known anti-alcoholism drug used to manage and treat alcohol dependence with little to no side effects, has been repurposed as a cancer therapy, according to multiple studies (Yip et al., 2011; Liu et al., 2013; Tawari et al., 2015). A combination between the DS and Cu raises intracellular copper levels, which leads to oxidative stress and ROS production, resulting in cell death (Boonstra and Post, 2004; Gupte and Mumper, 2009). DS in the bloodstream is quickly broken down to 2 molecules of diethyldithiocarbamate in serum, which augement the transfer of copper into cancer cells by acting as a potent chelator of transition divalent metal ions like Cu (Allensworth et al., 2015). The DS/Cu combination is a safe medication for repurposing because it is extremely cytotoxic to cancer cells but not to normal cells (Liu et al., 2014; Duanet al., 2014; Calderon-Aparicioal et al., 2015).

Figure 1 demonstrated that extracellular ROS in medium with DS/Cu and H_2O_2 produced more ROS than medium comprising of DDC-Cu, DS only, or Cu only. This suggests that the rapid cell death may be caused by ROS produced by DS plus Cu reactions. This finding of my research is in line with the earlier research by Allensworth et al., which reported that DS-Cu complex induces ROS generation. The results of the cell viability assay (Figure 2) showed that DS/Cu was highly cytotoxic to the two (2) resistant cell lines BT 549$G_{EM100nM}$ and MCF-7$_{5FU10\mu M,}$ whereas DS or Cu alone had little to no cyototoxic effects on the resistant cells. The anticancer effects of DS associated with Cu on cancer cells are further supported by these findings (Liu et al., 2013; Tawari et al., 2015; Tawari-Ikeh et al., 2017).

5. Conclusion

In redox processes, copper plays a critical role and initiates the production of reactive oxygen species. The formation of ROS by DS in conjunction with Cu is thought to be the reason of its high cytotoxic impact. The ROS induces oxidative stress that damages DNA, proteins, and lipids, resulting in cancer cell death. Consequently, an additional increase in ROS levels following exposure to DS/Cu may serve as a "Achilles heel" for cancer cells, ultimately resulting in their demise.

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