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In Vitro Cytotoxicity Effects of Encapsulated Disulfiram on Gemcitabine (dFdC) Acquired Resistant BT 549 Triple Negative Breast Cancer Cells

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Abstract

The prevalence of cancer is predicted to increase globally, making it one of the leading causes of mortality. The creation of novel and effective pharmacotherapeutics can increase cancer patients' chances of survival and lower their mortality rate. However, the significant cost and time involved in producing new anticancer medications make it difficult, and continuous use of these chemotherapeutic medications might lead to resistance. A practical alternative approach is drug repurposing, which involves using licensed medications with noteworthy pharmacokinetic and toxicological properties to treat different illnesses. It has been claimed that disulfiram (DS), a medication licensed to treat chronic alcoholism with few side effects, has anticancer action both *in vitro* and *in vivo*. However, DS's poor solubility in aqueous solutions and high instability in biological mediums limit its cytotoxic effect. The bioavailability of DS can be increased by using drug delivery vehicles. We created an encapsulated DS using polymeric micelles, and the results of our research revealed that the cytotoxic impact on the resistant cell line BT $549_{GEM100nM}$ was drastically enhanced because the half-life of DS in the encapsulated micelles was significantly extended.

Keywords: Disulfiram, Drug Repurposing, Resistant Cancer Cells, Cytotoxicity

1. Introduction

The health and socioeconomic systems are severely impacted by cancer, which is currently one of the world's leading causes of mortality. It is anticipated that the incidence of cancer will rise at an exponential rate (Sung *et al*., 2021) [15]. The main cancer treatment regimen, aside from surgery and radiation therapy is chemotherapy, which uses cytotoxic drugs to kill cancer cells, however this has serious side effects because these drugs can not distinguish cancer cells from rapidly dividing, growing normal cells (Bedard *et al*., 2020) [2].

Cancer stem cells (CSCs), a tiny population (0.5–1%) of quiescent, self-renewing cells capable of developing into the original tumors, provide a significant problem for cancer treatment due to tumor resistance to these cytotoxic medications (Li *et al*., 2009) [10]. According to Al-Hajj *et al*. (2003) [1], these CSCs are the primary population of tumor cells with high expression of cell-surface markers, such as increased ALDH + activity and an enriched CD44 high/CD24 low subfraction of cells. They are thought to be the primary cause of chemotherapy failure and drug resistance.

The process of developing new drugs is expensive, costing billions of dollars, takes a long time to finish, and has a very overwhelming failure rate of up to 95% (Bertolini *et al*., 2015) [3]. Drug repurposing, the process of finding novel therapeutic applications for already-approved or existing medications, has gained popularity in recent years.

It has been reported that disulfiram (DS), a first-line anti-alcoholism medication used for more than 60 years (Suh *et al*., 2006) [16], exhibits anticancer action both *in vitro* and *in vivo* (Yip *et al*., 2011) [23]. According to reports, DS's anticancer impact is dependent on copper (Cu), and the DS/Cu combination is a potent inducer of reactive oxidative stress (ROS) and an inhibitor of the proteasome–NF-κB pathway (Schreck *et al*., 1992) [14]. Therefore, DS functions as a ROS scavenger and can specifically reduce ALDH activity, a functional CSC marker. Due to its high instability in biological media and poor solubility in aqueous

solutions, which results in poor bioavailability, DS's use as a potential anticancer drug for clinical administration is severely restricted (Eneanya *et al*., 1981) [7]. Chemotherapeutic drugs have been shown to be more effective when used in drug delivery systems that use nanocarriers like nanoparticles, liposomes, nanospheres, PLGA, and polymeric micelles to deliver drugs selectively into tumor cells and increase their permeability across cells (Torchilin, 2007) [20]. We created an enclosed DS using polymeric micelles, and our study's findings showed that the DS's half life in the encapsulated micelles was greatly extended (Tawari-Ikeh *et al*., 2017) [19].

1.1 Objective and Aim of the Study

One of the main obstacles to effectively treating cancer cells is the development of chemotherapeutic drug resistance in initially sensitive cancer cells. The purpose of the study was to assess the drug sensitivity of encapsulated DS in polymeric micelles *in vitro* on BT 549 breast cancer cell line that had developed resistance to gemcitabine (dFdC).

2. Methods

2.1 Cell Lines and Reagents

The resistant cell line BT $549_{GEM100nM}$ was created by continuously cultivating the parental cell lines in a medium incorporating Gemcitabine (dFdC) (Sigma, Dorset, UK) in a stepwise concentration increasing procedure (Tawari Erebi Patricia, 2024) [17]. The parental cell line BT 549 was acquired from ATCC, Middlesex, UK. Sigma (Dorset, UK) provided disulfiram (DS), polymericmicelles, copper chloride (CuCl2), and 99.9% dimethylsulfoxide (DMSO); Lonza (Wokingham, UK) provided DMEM and fetal calf serum (FCS). The solvent evaporation approach was used to create the DS-loaded micelles (Tawari-Ikeh *et al*., 2017) [19].

2.2 Cell Culture and Cytotoxicity Analysis

Dulbecco's modified Eagle's medium (DMEM) (Lonza, Wokingham, UK) supplemented with 10% FCS, 50 units ml_1 penicillin, and 50 mgml_1 streptomycin was used to cultivate the cell lines. The medium containing 100nM of GEM was used to sustain the BT $549_{GEM100nM}$ cells. The overnight grown cells (5 X 10^3 per well) in 96-well flat-bottomed microtiter plates were treated to DS, DS-Cu, and PMDS-Cu for 72 hours before being subjected to a standard MTT assay for the *in vitro* cytotoxicity test (Plumb *et al*, 1989).

3. Results

3.1 *In Vitro* Release Studies

Figure 1 shows the cumulative release profile of free DS and DS from PM in PBS (containing 0.5% Tween 80 (w/v) at acidic pH 5 and pH 7.4). The release of DS in acidic conditions was higher than that of alkaline conditions, indicating that DS can be released in greater quantities within the acidic tumor microenvironment than in blood at a physiological pH of 7.4. In both acidic and alkaline environments, PM can release DS. While the discharge of DS from PM persisted for more than 25 hours, the degradation of free DS happened quickly.

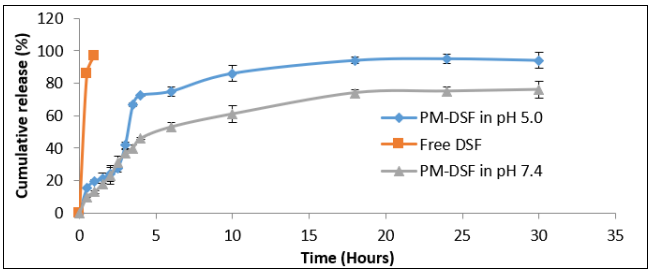

Fig 1: Represenative Plots of *In vitro* cumulative release of DS from PM in PBS (containing 0.5% tween 80) at pH 5 and 7.4

(DS –disulfiram, PM – polymeric micelles, PBS: phosphate buffer saline).

3.2 *In Vitro* Half-Life of Disulfiram in Horse Serum

Free DS was speedily broken down to indiscernible levels in a matter of minutes, whereas DS was slowly discharged from micelles with a half-life of almost three hours, according to the *in vitro* release profile of free DS and PMDS distributed in horse serum (Figure 2). While the release of DS from PM persisted for more than three hours, the degradation of free DS happened quickly. Furthermore, compared to free DS, PMDS which has a far longer half-life, the DS from PM nanoparticles was released much more slowly.

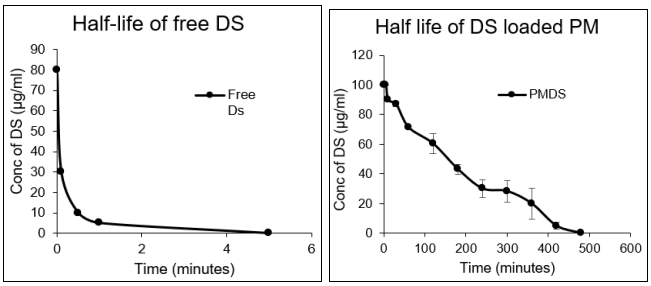

Fig 2: Democratic Plots of *In vitro* half-life of free DS and DS loaded PM in horse serum

The half-life of free DS and DS loaded micelles measured at interval time points using HPLC. (DS: disulfiram, PM: polymeric micelle)

3.3 Cell Viability Assay of DS, Free DS-Cu and PMDS-Cu on Gemcitabine-Resistance Cancer Cell Line

Information from the investigation of MTT cytotoxicity in the BT549 Gemcitabine-resistant cancer cell line (BT $549_{GEM100nM}$), Figure 3 revealed that the *in vitro* cytotoxicity of PMDS supplemented with Cu (1µM) was similar to that of free DS/Cu, but free DS had little or no cytotoxic impact.

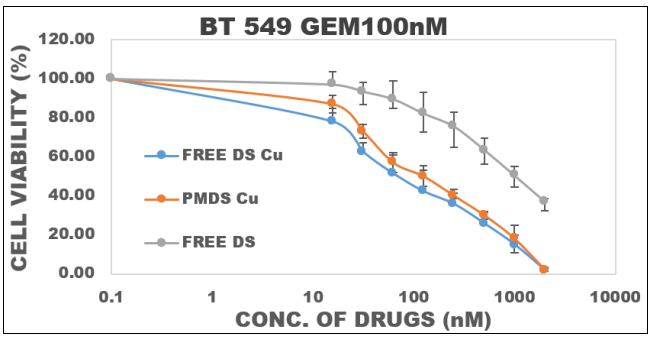

Fig 3: Democratic Drug Concentration Response Curves of Free DS, DSCu and PMDS Cu.

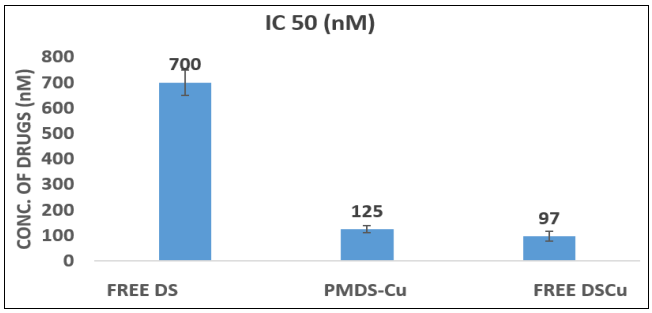

Fig 3B: Representative Histograph of Free DS, DSCu and PMDS Cu

4. Discussion

One of the biggest obstacles to treating and managing patients with breast cancer is still cancer resistance to traditional anticancer medications (Gottesman, 2002) [9]. Drug repositioning is necessary since, despite recent advances in drug research, very few clinically effective oncology medications ever make it to market (Walker and Newell, 2009) [22]. Disulfiram also called as Antabuse, has been utilised to treat alcoholism since the 1940s (Chen *et al*., 2006) [5]. However, in recent years, it has been shown to be highly cytotoxic to many cancer cell types *in vitro* (Liu *et al*., 2014, Calderon-Aparicioal *et al*., 2015) [12, 4]. According to certain research, DS targets CSCs and makes a number of cancer types more susceptible to traditional anticancer medications (Dinarvand *et al*., 2011; Triscott *et al*., 2015) [6, 21].

However, there are still certain obstacles in the way of translating DS from the lab to the clinic. The current oral disulfiram formulation has a half-life of less than four minutes and is rapidly eliminated in the bloodstream due to its extreme instability in an acidic stomach environment (Liu *et al*., 2014) [12]. Recently, we created an encapsulated DS in polymeric micelles (PM) forming the PMDS (Tawari-Ikeh *et al*., 2017) [19]. DS can be discharged from PM under both acidic and alkaline conditions, according to the study's *in vitro* release results (figure 1). In comparison to the release in blood at a physiological pH of 7.4, the release of DS in acidic settings was greater than that in alkaline conditions, indicating that DS can be released in larger quantities within the acidic tumor microenvironment. Furthermore, the half-life of DS in the encapsulated micelles was considerably extended (Figure 2) from a few minutes in free DS to more than three hours in the PMDS, which can shield the DS from rapid degradation and prolong its presence in the bloodstream, increasing the likelihood of sustained release of DS into the tumor site.

The MTT data's cell viability curve (Figure 3) revealed variations in the dosage response of Free DS, DS-Cu complex, and PM-DS supplemented with Cu (1µM). In the BT549 Gemcitabine-resistant cancer cell line (BT $549_{GEM100nM}$), the *in vitro* cytotoxicity of PMDS supplemented with Cu (1µM) was comparable to that of free DS/Cu, but free DS alone showed little to no cytotoxic impact. These results are consistent with previous research showing that DS-cu is cytotoxic to cancer cells (Liu *et al*., 2013; Tawari *et al*., 2015; Tawari-Ikeh *et al*., 2017) [11, 18, 19].

5. Conclusion

Encapsulated DS in polmeric micelles (PMDS) can effectively be exploited for clinical application in the delivery of disulfiram into tumour cells. This formulation can forestall the fast degradation of disulfiram in gastric environment and blood system which can provide a sustained release of this drug into tumour cells.

6. References

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