MGMT Inhibition by Disulfiram Sensitizes ER+ Breast Cancer Cells to Temozolomide and Cyclophosphamide

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PROBLEM

MGMT (O6-alkylguanine DNA methyltransferase), a DNA repair protein leading to chemoresistance, is overexpressed in a majority of cancers, including breast cancer. MGMT expression directly correlates with ER expression and tamoxifen resistance, making ER positive breast cancer resistant to alkylators like Temozolomide and Cyclophosphamide. Alkylhydrazine Dealkylase (ALDH) activity, as a survival/proliferation cell marker, has also been reported to inversely correlate with MGMT expression in other cancers, has also been linked to chemotherapy and radiation resistance.

BACKGROUND

In breast tumors, MGMT expression is elevated at levels that are 3-4 fold higher than in the normal breast (2, 3). MGMT has been recognized as a central determinant of tumor resistance to alkylating agents and remains an important target for inhibition development (2). Overexpression of MGMT is associated with the loss of Disulfiram (DSF), an MGMT inhibitor, either alone or in combination with alkylators (Temozolomide/Cyclophosphamide) in the treatment of breast cancer. Disulfiram (DSF), a thiosemicarbazone chlorophenol, also known as Antabuse, is a carbamide derivative clinically used for treating alcoholics. DSF is a relatively non toxic oral drug (6), Temozolomide (TMZ), Temozolomide, an alkylating agent, with activated diterate, only slightly biodegradable, has demonstrated antineoplastic activity against a broad range of tumor types (gastro, melanoma, non-small cell lung cancer, and carcinomas of the esophagus, and liver) (5, 6). Temozolomide is an alkylating agent with dose dependent biological activity which has been for decades successfully used to treat a variety of cancers. At high doses, it is associated with increased cytotoxicity and immunosuppression, while at low, continuous doses, it shows immunomodulatory and antitumorigenic properties (9). These observations paved the way to investigate the effect of alkylators such as Temozolomide/Cyclophosphamide in presence or absence of MGMT blockers (DSF) in breast cancer cells.

OBJECTIVE

To investigate whether disulfiram, either alone or in combination with Temozolomide or Cyclophosphamide can inhibit breast cancer growth and establish whether use of DSF as MGMT inhibitor would allow for lower doses of Temozolomide or Cyclophosphamide.

METHODS

We have tested the effect of Antabuse (Disulfiram, DSF), as a dual MGMT and ALDH inhibitor, at various doses, in combination with Temozolomide (TMZ) or etoposide (VP-16) on ER+ breast cancer cells.

RESULTS

Effect of Disulfiram on Normal Breast Epithelial Cells and Breast Cancer Cells: Normal breast epithelial cells (MCF10A) and 1640, T47D and ZR75 cells treated with different concentrations of DSF. Forty eight hour post treatment cell viability assays were performed. Results reveal that DSF has minimal effect on normal breast epithelial cells (MCF10A) whereas ER A positive breast cancer cell growth was dose dependently inhibited (Figure 1).

Pharmacological Cytotoxicity Assays: MCF7 breast cancer cells treated with different concentrations of DSF and 48 hr post treatment cell viability was assessed and ALDH activity was measured. Results reveal that DSF dose dependently decreased ALDH activity in breast cancer cells. In another experiment, MCF7 cells treated with single agents (DSF, Cyclophosphamide and Temozolomide) in combination of these drugs and 48 hr post treatment ALDH activity was measured. Results reveal that single agents DSF, Cyclophosphamide and Temozolomide decreased ALDH activity compared to control and combination therapy further decreased (Figure 2).

Combination Therapeutic Effect on Various Assay Combos: We used colony formation assay to determine the effectiveness of these drug treatments on breast cancer cells. We plated breast cancer cells (MCF10A, T47D, ZR75) in 6 well plates and treated with Temozolomide, Cyclophosphamide and OSF alone or in combination. Results revealed that DSF inhibited the colony formation of these cells and DSF in combination with Temozolomide significantly decreased the colony formation of these cells (Figure 3).

Combination Therapeutic Effect on Osteoclast Colony Formation: We used colony formation assay to determine the effectiveness of these drug treatments on breast cancer cells. We plated breast cancer cells (MCF10A, T47D, ZR75) in 6 well plates and treated with Temozolomide, Cyclophosphamide and OSF alone or in combination. Results revealed that DSF inhibited the colony formation of these cells and DSF in combination with Temozolomide significantly decreased the colony formation of these cells (Figure 4).

CONCLUSIONS

DSF at very low doses (achievable in human serum with standard DSF clinical dosing) decreases ER+ breast cancer cell growth (MCF10A, T47D and ZR75) in a dose dependent manner.

DSF further sensitizes breast cancer cells to TMZ or etoposide or significantly inhibits breast cancer growth without causing unwanted side effects on the normal breast epithelial cells.

DSF alone or in combination with TMZ (DSF + TMZ) and/or OSF (DSF + OSF) significantly inhibits expression of MGMT, alkylhydrazine dealkylase, Fas and FasL gene products (survivin) – all important in tumor proliferation and survival.

DSF alone or in combination with TMZ (DSF + TMZ) and/or OSF (DSF + OSF) significantly inhibits apoptosis in breast cancer cells.

In a dose dependent manner, DSF inhibited colony formation, effect which was further enhanced by addition of TMZ (DSF + TMZ).

Similar, DSF alone or in combination with TMZ (DSF + TMZ) and/or OSF (DSF + OSF) decreased the metastatic potential of breast cancer

REFERENCES


