

Chemosensitizing and cytotoxic effects of 2-deoxy-D-glucose on breast cancer cells

ABSTRACT

Background: Accelerated glucose uptake for anaerobic glycolysis is one of the major metabolic changes found in malignant cells. This property has been exploited for imaging malignancies and as a possible anticancer therapy. The nonmetabolizable glucose analog 2-deoxyglucose (2DG) interferes with glucose metabolism leading to breast cancer cell death.

Aims: To determine whether 2DG can synergize with chemotherapeutic agents commonly used in breast cancer treatment and identify cellular characteristics associated with sensitivity to 2DG.

Materials and Methods: SKBr3 breast cancer cells were incubated with varying concentrations of 5-fluorouracil (5FU), doxorubicin, cisplatin, cyclophosphamide, or herceptin with or without 2DG. Cell viability was measured using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay.

Results: Combining 2DG with doxorubicin, 5FU, cyclophosphamide, and herceptin resulted in enhanced cell death compared with each agent alone, while in combination with cisplatin, the amount of cell death was additive. Mouse embryo fibroblasts (MEF) mutated for p53 (-/-) were 30% more sensitive to the cytotoxic effects of 2DG than the parental cell lines. Cells mutated for Bax/Bac, genes involved in protection from apoptosis, are slightly more sensitive than the parental cell lines.

Conclusions: These results indicate that 2DG acts synergistically with specific chemotherapeutic agents in causing cell death and the class of chemicals most sensitive appear to be those which cause DNA damage.

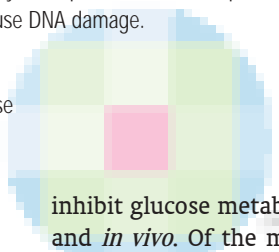
KEY WORDS: Breast cancer, chemotherapy, 2-deoxy-D-glucose

INTRODUCTION

Therapeutic selectivity is one of the most important considerations in cancer chemotherapy. The design of therapeutic strategies to preferentially kill malignant cells while minimizing harmful effects on normal cells depends on our understanding of the biological differences between cancer and normal cells. Accelerated glucose uptake for anaerobic glycolysis is one of the major metabolic changes found in malignant cells, and experimental conditions of glucose deprivation have been demonstrated to cause cell death in many transformed cell lines.^[1,2] Multiple related mechanisms may be involved in glucose-deprivation-induced signaling including the activation of kinases,^[3] changes in the redox state of the cell, or generation of free radicals.^[4]

Glucose analogs have been found to profoundly

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inhibit glucose metabolism in cancer cells *in vitro* and *in vivo*. Of the many glucose analogs which have been investigated, 2-deoxy-D-glucose (2DG) has been proven effective in the inhibition of glucose metabolism and ATP production. 2DG is a structural analog of glucose differing at the second carbon atom by the substitution of hydrogen for a hydroxyl group and appears to selectively accumulate in cancer cells by metabolic trapping due to increased uptake, high intracellular levels of hexokinase, and low intracellular levels of phosphatase.

We and others have reported that treatment of breast cancer cells with 2DG causes cell death.^[5,6] In combination with radiation treatment, 2DG at non-toxic doses acts as a radiosensitizer in various cancer cell types.^[7,8] In this study, we have determined the effect of 2DG on the cytotoxic effect of chemotherapeutic agents commonly used in breast cancer treatment and explored characteristics of cells which may enhance the cytotoxicity. Our results demonstrate that 2DG acts synergistically with specific chemotherapeutic agents in causing cell death and the class of chemicals most sensitive consists of those which cause DNA damage.

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MATERIALS AND METHODS

All cell lines were obtained from ATCC and were maintained in medium supplemented with 10% heat inactivated fetal bovine serum, penicillin (100 units per ml), streptomycin (100 µg per ml), and amphotericin B (0.25 µg per ml) in a humidified atmosphere of 5% CO₂ and 95% O₂ at 37°C. Cells were seeded at 6000-10,000 cells per well in a 96-well plate at least 1 day prior to use for all experiments. Media containing 2DG or other chemicals were added as described in the text. Nowhere in the text, it is mentioned.

For viability assays, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (0.5 mg per ml in PBS) was added to each well, after the completion of treatment with the experimental compounds, and incubation continued at 37°C for an additional 3 h. The medium was then discarded and 200 µl of DMSO was added to each well to solubilize the colored formazan product. Absorbance was read at 570 nm on a scanning microtiter spectrophotometer plate reader after agitating the plate for 5 min on a shaker. The data are expressed as absorbance relative to untreated cells in the same experiment and standardized to 100%. All data points were performed in triplicate. Results are the average of two to three experiments.

RESULTS

SkBr3 human breast cancer cells were incubated with varying concentrations of 5FU, doxorubicin, cisplatin, cyclophosphamide, or Herceptin for 20 h, subsequently incubated for an additional 4 h with 500 µM 2DG. Cell viability was measured using the MTT assay. Concentrations of DG and drug as well as incubations times were varied to obtain the

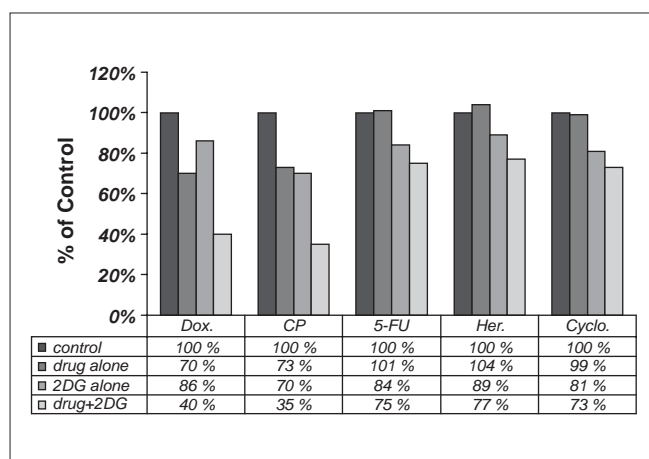


Figure 1: Summary of drug combinations with 2DG SKBr3 cells incubated with the indicated agent for 24 h. 2DG was added and incubation continued for 4 h. Cytotoxicity was evaluated using the MTT assay. 2DG at 0.5 mM was used for all combinations except cisplatin (1 mM 2DG). The concentrations of the chemotherapeutic agents were doxorubicin (dox), 1 µM; cisplatin (CP), 20 µM; 5-fluorouracil (5-FU), 20 µM; Herceptin (Her); cyclophosphamide (cyclo), 2 mM

maximum effect on cells. The results of these experiments are summarized in Figure 1. A greater than additive effect on cell cytotoxicity is observed with 2DG in combination with doxorubicin, 5FU, Herceptin, and cyclophosphamide. An additive effect is seen with cisplatin.

Results for p53^{-/-} and Bax/bac are not mentioned.

DISCUSSION

2DG has been studied extensively in tissue cultures, animals, and patients as a possible targeted therapeutic agent to treat cancer or enhance the effect of other treatment modalities.^[9] Breast cancers, like other cancers, are dependent on increased glucose uptake to sustain cell growth. We have previously demonstrated that 2DG can synergize with radiation therapy in causing breast cancer cell death. We have now examined the effect of 2DG in combination with chemotherapeutic agents commonly used on breast cancer treatment.

In our studies, we observed that 2DG enhances the effects of two agents which are known to act on DNA, doxorubicin and 5FU (the Figure shows different). Doxorubicin, a member of the anthracycline family, is known to cause generation of intracellular superoxide and hydrogen peroxide, which can mediate mitochondrial damage and apoptosis in a p. 53-independent manner. We and others have found that 2DG treatment results in increased production of reactive oxygen species. Therefore, it is not surprising that 2DG enhances the cytotoxic effect of doxorubicin. We did not observe enhanced cytotoxicity of 2DG with cisplatin in breast cancer cells, though this combination enhanced cytotoxicity in head and neck cancers.

SkBR3 cells overexpress c-erb/Her-2 and thus we anticipated a robust cytotoxic effect of Herceptin on these cells. In our cell culture system, we observed very little cytotoxicity with Herceptin alone. However, in combination with 2DG, the amount of observed cytotoxicity doubled. Using two selective agents, 2DG in combination with Herceptin, may provide an effective therapy for those patients who are marginally sensitive to the effects of Herceptin.

There are several limitations to our study. We used a single cell line for testing these agents and it is well established in the breast cancer field that the biological breast subtypes respond differently to chemotherapy. Therefore, these results need to be repeated in estrogen receptor-positive and-negative cell lines as well as in animal models.

From these results we propose that 2DG may be a good chemosensitizer for chemo-resistant patients since it alters ROS or redox state and sensitizes the cells to further damage caused by chemo agents.

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