Glucose deprivation-induced metabolic oxidative stress and cancer therapy

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Abstract
Cancer cells (vs. normal cells) demonstrate evidence of oxidative stress, increased glycolysis, and increased pentose cycle activity. The oxidative stress in cancer cells has been hypothesized to arise from mitochondrial dysfunction leading to increased levels of hydroperoxides, and cancer cells have been proposed to compensate for this defect by increasing glucose metabolism. Glucose metabolism has also been shown to play a role in hydroperoxide detoxification via the formation of pyruvate (from glycolysis) and NADPH (from the pentose cycle). Furthermore, in cancer cells, glucose deprivation as well as treatment with 2-deoxyglucose (2DG) has been shown to induce oxidative stress and cytotoxicity. Additionally, transformed cells have been shown to be more susceptible to glucose deprivation (and 2 DG)-induced cytotoxicity and oxidative stress than untransformed cells. These results support the hypothesis that cancer cells have a defect in mitochondrial respiration leading to increased steady state levels of O$_2^-$ and H$_2$O$_2$, and glucose metabolism is increased to compensate for this defect. The application of these findings to developing cancer therapies using 2DG combined with inhibitors of hydroperoxide metabolism to induce radio/chemosensitization is discussed, as well as the possibility that FDG-PET imaging may predict tumor responses to these therapies.

Keywords
L-buthionine-[S,R]-sulfoximine; chemotherapy; cisplatin; F-2-deoxy-D-glucose coupled with positron emission tomography; glucose deprivation; glutathione; glycolysis; hydroperoxides; NAC; oxidative stress; 2-deoxy-D-glucose

2-Deoxy-D-glucose and metabolic oxidative stress in cancer cells
It is known that cancer cells take up and metabolize glucose to a greater extent than normal cells.[1] We have shown that glucose deprivation preferentially results in cytotoxicity via mechanisms involving oxidative stress in transformed cells, relative to normal cells.[2,3] Cancer cells are believed to exist under a chronic condition of metabolic oxidative stress compared to normal cells, and cancer cells may upregulate glucose metabolism to produce more pyruvate and NADPH to protect against hydroperoxide-induced toxicity.[2,3] If this were true, depriving cancer cells of glucose would be expected to inhibit pathways used to protect against increased steady-state levels of hydroperoxides resulting in selective cytotoxicity and...
sensitization of cancer cells to agents thought to induce oxidative stress, relative to normal cells. This biochemical rationale forms the theoretical underpinnings of the preclinical studies using 2-deoxy-D-glucose (2DG) to enhance cancer therapy discussed in the following sections of this review.

Although it is not possible to totally deplete cancer cells of glucose in vivo, it is possible to treat animals and humans with 2DG. 2DG is a relatively nontoxic analog of glucose that competes with glucose for uptake via glucose transporters as well for being phosphorylated by hexokinase (as is glucose) at the entry point to glycolysis. The competition between 2DG and glucose is thought to cause inhibition of glucose metabolism, thereby creating a chemically induced state of glucose deprivation. Although there are reports that the phosphorylated form of 2DG (2DG-6-P) can proceed through the first step in the pentose cycle via glucose 6-phosphate dehydrogenase, leading to the regeneration of one molecule of NADPH,[4] it appears to be incapable of further metabolism in the pentose cycle as well as incapable of metabolism to form pyruvate. The administration of 2DG to mice has been shown to be effective in the inhibition of glucose metabolism without causing toxicity until very high levels are achieved (LD50 ≥ 2 g/kg body weight).[5] Therefore, using 2DG as an inhibitor (albeit not complete) of glucose metabolism in vivo may provide a relatively nontoxic and effective addition to multimodality cancer therapies designed to limit hydroperoxide detoxification and selectively target cancer cells for the purpose of enhancing radio-and chemosensitivity in cancer therapy.

2-Deoxy-D-glucose-induced oxidative stress and radiosensitization

2DG has been known for many years to be cytotoxic to cancer cells as well as to enhance the cytotoxicity of ionizing radiation toward cancer cells in culture, animals, and clinical trials. [6,7-10] Experiments to determine if oxidative stress could be contributing to 2DG-induced cytotoxicity and radiosensitization in human cervical carcinoma cells (HeLa) as well as untransformed (208F) versus oncogene transformed (v-fos) rat fibroblasts (FBJ/R)[10] have been accomplished. The results demonstrated that glucose deprivation using increasing concentrations of 2DG (4, 6, 8 mM) for 8 h in HeLa cells caused significant cytotoxicity using a clonogenic survival assay.[10] At every dose of 2DG tested, co-incubation with 30 mM NAC (a thiol antioxidant) inhibited 2DG-induced clonogenic cell killing in HeLa cells, suggesting disruptions in thiol metabolism contributing to toxicity.[10] NAC also inhibited 2DG-induced radiosensitization seen in HeLa cells exposed to 4 Gy and 8 Gy of ionizing radiation (IR) following 16 h in the presence of 6 mM 2DG.[10] Exposure to 6 mM 2DG for 8 h was also found to cause disruptions in glutathione metabolism in HeLa cells characterized by a loss in total glutathione content, and 30 mM NAC treatment was found to reverse these effects.[10] Additionally, it was shown that 2DG (6 mM and 10 mM) was more cytotoxic to oncogene-transformed cells (FBJ/R), relative to the untransformed (208F) cells. Furthermore, 6 mM 2DG for 8 h induced selective radiosensitization with 2Gy in oncogene-transformed cells (FBJ/R), relative to untransformed (208F) cells.[10] These results supported the hypothesis that transformed cells were more susceptible to 2DG-induced cytotoxicity and oxidative stress as well as 2DG-induced radiosensitization, compared to untransformed cells.

2-Deoxyglucose in combination with BSO-induced glutathione (GSH) depletion

The hypothesis that inhibiting hydroperoxide detoxification could enhance cancer cell killing with 2DG was investigated with L-buthionine-[S,R]-sulfoximine (BSO). BSO is a drug being tested in clinical trials for cancer therapy that reversibly inhibits glutamate cysteine ligase (GCL) activity, therefore inhibiting GSH synthesis and resulting in GSH depletion and chemosensitization.[11] GCL is the rate-limiting enzyme in GSH synthesis,[11-15] which
participates in the detoxification of hydroperoxides, and we hypothesized that GCL activity
would be up-regulated by cancer cells treated with 2DG in an attempt to compensate for
increased 2DG-induced metabolic oxidative stress.[15] Additionally, it was proposed that if
the GCL activity protected against 2DG induced metabolic oxidative stress, then combining
BSO treatment with 2DG should increase 2DG-induced cytotoxicity as well as endpoints
indicative of metabolic oxidative stress in cancer cells.[15] In support of this hypothesis,
treatment with 20 mM 2DG in MDA-MB231 human breast carcinoma cells resulted in
significant cell killing over 24 and 48 h while increasing steady-state levels of GCL mRNA as
determined by real-time PCR, GCL activity assays, and glutathione content.[15] Furthermore,
the inhibition of the GCL activity with 1 mM BSO further sensitized the cells to 2DG while
increasing the GSSG percentage (%GSSG) and measurements of steady-state pro-oxidant
levels using the oxidation sensitive dye.[15] These results demonstrate that combining 2DG
with BSO significantly enhanced cell killing by 2DG, suggesting that the inhibition of
hydroperoxide detoxification via the GSH-dependent peroxidases inhibited the induction of
this protective pathway.[15] These results provided the first clear evidence that inhibitors of
hydroperoxide metabolism could represent a significant target for enhancing the effectiveness
of cancer therapies based on 2DG-induced toxicity and metabolic oxidative stress in human
breast cancer cells.

2-Deoxyglucose in combination with cisplatin

Cisplatin (cis-diamminedichloroplatinum(II)) is an effective antitumor agent and is one of the
most widely used drugs either alone or in combination with other chemotherapeutic agents or
with radiotherapy in the management of locally advanced or recurrent squamous cell
carcinomas of the head and neck.[16] There have been barriers to the use of cisplatin in the
clinical setting because of the occurrence of severe toxic side effects, which include nephro-
and ototoxicity, nausea and vomiting,[17] and cisplatin resistance.[18] Cisplatin is classified
as an alkylating agent, and its efficacy is believed to be a function of cisplatin-DNA adducts
inhibiting DNA replication and transcription, ultimately resulting in cell death.[19] More
importantly, several studies have shown that cisplatin-induced cytotoxicity may be related to
the inhibition of the thioredoxin reductase activity which participates in important cellular
defense systems that protect against oxidative stress induced by hydroperoxide production in
cancer cells.[20-23] Since 2DG has been previously shown to disrupt glutathione metabolism,
which also participates in hydroperoxide metabolism,[10,15] we hypothesized that the
combination of 2DG and cisplatin would enhance cancer cell killing by mechanisms involving
oxidative stress. Because cisplatin is the most commonly used drug in the management of
locally advanced or recurrent head and neck cancer,[16] we conducted experiments using FaDu
human head and neck cancer cells.[24] We found that the combination of 20 mM 2DG and 0.5
μM cisplatin showed at least additive (and possibly synergistic) cell killing in FaDu human
head and neck cancer cells (determined by the clonogenic assay) compared to 2DG or cisplatin
alone.[24] Treatment with 2DG and cisplatin also caused perturbations in parameters indicative
of oxidative stress, including decreased intracellular total glutathione and increased percentage
of glutathione disulfide (%GSSG). The increase in %GSSG induced by 2DG + cisplatin
suggested that oxidative stress was involved.[24] We speculated that the combination of 2DG
+ cisplatin might have resulted in increased steady-state levels of hydroperoxides and this
increase might have exceeded the metabolic capabilities of the glutathione peroxidase and
glutathione reductase system to maintain glutathione in the reduced form. Although we did not
measure thioredoxin levels, it is possible that oxidized thioredoxin was also increased in
response to treatment with 2DG and cisplatin. In support of this notion, 15 mM NAC was
shown to significantly increase total glutathione, inhibit the increase in %GSSG in 2DG- and/
or cisplatin-treated cells, and inhibit the cytotoxicity induced by 2DG + cisplatin.[24]
To further probe the involvement of thiol and hydroperoxide metabolism in the cytotoxicity of 2DG and cisplatin in FaDu human head and neck cancer cells, BSO was used in combination with 2DG and cisplatin. We found that 1 mM BSO enhanced the toxicity of 2DG and cisplatin, when used alone or in combination. Furthermore, thiol analysis shows that BSO depleted total glutathione in all groups and increased the %GSSG significantly in both the 2DG and 2DG + cisplatin groups. These results are consistent with the aforementioned hypothesis that BSO enhances 2DG + cisplatin-induced oxidative stress by limiting hydroperoxide detoxification via the glutathione/glutathione peroxidase system. Furthermore, the effects of BSO on 2DG + cisplatin-induced cytotoxicity and %GSSG were reversed with NAC treatment. These results strongly support the hypothesis that the combination of 2DG and cisplatin enhances metabolic oxidative stress and cytotoxicity in human head and neck cancers that can be enhanced by inhibiting GSH-mediated hydroperoxide metabolism. Furthermore, since the thiol antioxidant (NAC) inhibited both the cytotoxicity associated with 2DG + cisplatin + BSO, as well as the parameters indicative of oxidative stress, these results strongly support a causal relationship between 2DG + cisplatin-induced oxidative stress and enhanced cancer cell killing. These observations have now been extended to studies of FaDu cells growing as human tumor xenografts in nude mice and it was found that 2DG enhanced antitumor responses mediated by cisplatin and cisplatin combined with ionizing radiation. Since chemoradiotherapy with cisplatin has emerged as the standard of care for locally advanced unresectable tumors and for postoperative adjuvant therapy in patients with cancer of the head and neck, these results suggest that 2DG could be an excellent adjuvant to the current standard of care for head and neck cancer patients.

2-Deoxyglucose in combination with other chemotherapeutic agents

The promising results observed with 2DG in combination with inhibitors of hydroperoxide detoxification (BSO and cisplatin) provide an opportunity to investigate other chemotherapeutic agents that increase hydroperoxide or ROS production. For instance, ROS has been found to be involved in the toxicity of taxol (paclitaxel), which is a commonly used chemotherapeutic agent for the treatment of breast carcinomas. Alexandre et al. 2006 reported that exposure of A549 human lung cancer cells to 0.1-5 μM taxol for up to 15 min resulted in a concentration-dependent intracellular increase in O$_2^•$- and H$_2$O$_2$ levels. In this work, NAC and glutathione protected the cells from taxol-induced cytotoxicity while BSO significantly increased paclitaxel cytotoxicity and H$_2$O$_2$ accumulation. This work suggested that H$_2$O$_2$ production and accumulation is an important mechanism responsible for taxol-induced cytotoxicity and makes this agent an excellent candidate to be explored in combination with 2DG in breast cancer models.

FDG-PET as a predictor of response to chemotherapy

Fundamental differences in glucose metabolism between transformed and normal cells are used clinically for imaging cancerous tissues using tracer amounts of F-2-deoxy-D-glucose (FDG) coupled with positron emission tomography (FDG-PET). Studies have shown that there may be strong correlations between glucose uptake, glycolysis, and treatment resistance in some tumors. Specifically, tumors with a lower FDG uptake tend to respond better to treatment than those with a higher FDG uptake. This suggests that new, relatively nontoxic adjuvants to chemoradiotherapy are needed to treat tumors with a high FDG uptake. FDG-PET is now a standard test in various cancer disease sites especially HNSCC for staging pretreatment, surveillance or restaging posttreatment. In addition, the degree of glucose metabolic derangement, as assessed by the FDG-PET uptake has a prognostic value, as several groups have reported worse survival for patients with tumors showing a high pretreatment FDG uptake. Our group has hypothesized that cancer cells increase their utilization of glucose in order to offset increased steady-state levels of hydroperoxides.
produced by defective mitochondrial oxidative metabolism. Because FDG-PET can be used as a noninvasive in vivo index of glucose utilization, tumors with a high FDG uptake may represent tumors with high metabolic production of hydroperoxides and thus increased susceptibility to 2DG-induced radio-/chemosensitization. Using this logic, we predict that 2DG should sensitize tumors with a greater FDG uptake to a greater extent to anticancer agents that further increase hydroperoxide production and metabolic oxidative stress, relative to low FDG-uptake tumors. Following the same logic, we propose that the relative increase of the pretreatment FDG uptake could be proportional to the amount of 2DG-induced chemosensitization. In this regard, pretreatment FDG-PET scans could be used clinically to predict tumor susceptibility to combined modality cancer therapies utilizing 2DG in combination with inhibitors of hydroperoxide metabolism as relatively nontoxic (to normal tissue) adjuvants to conventional therapies thought to increase oxidative stress. In support of this hypothesis two different human xenograft tumors grown in nude mice that differed in FDG uptake showed that tumors that took up more FDG also responded significantly better to the antitumor effects of 2DG + cisplatin.[27]

SUMMARY AND CONCLUSIONS

Compelling evidence is beginning to accumulate suggesting that cancer cells increase glucose metabolism in order to increase the production of pyruvate and NADPH to detoxify excessive hydroperoxide production due to defective mitochondrial oxidative metabolism.[3] Cancer cells appear to rely heavily on glucose metabolism for survival in this prooxidant state, which makes glycolytic inhibition combined with the inhibition of hydroperoxide detoxification an extremely attractive new strategy for selectively killing cancer cells and sensitizing them to therapeutic interventions, relative to normal cells. Finally, the integration of 2DG into the therapy of head and neck cancer exploits this theoretical rationale and has the advantage of being linked to imaging assessment where pretreatment and FDG-PET can be seamlessly integrated as a predictor of therapeutic responses.

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