Targeting glucose metabolism with 2-deoxy-D-glucose for improving cancer therapy

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“Appropriate combinations of tumoricidal agents such as ionizing radiation and chemotherapeutic drugs with 2-DG provide unique opportunities to selectively destroy tumors, reduce toxicity to normal tissues and significantly enhance the therapeutic efficacy.”

Tumors require enhanced glucose usage to generate metabolic energy (ATP) and building blocks for macromolecular synthesis in order to sustain rapid cell proliferation. This manifests in increased dependency on the glycolytic pathway (even in the presence of oxygen) for energy (ATP) supply as compared with surrounding normal tissues, a phenomenon first described almost 80 years ago by Otto Warburg [1], who considered this altered metabolic state to be a hallmark of cancer causally associated with the very process of carcinogenesis [2].

The enhanced glucose usage and glycolysis emerging concomitant with malignant transformation, mitochondrial malfunction (linked to or independent of mutations), microregional hypoxia and evolution of aerobic glycolysis in most types of human tumors [3,4] constitute key factors contributing to tumorigenesis and should, therefore, form effective targets for cancer diagnosis, treatment and prevention. While the enhanced glucose uptake is being increasingly exploited for the noninvasive detection and grading of tumors by positron emission tomography using the F-18 labeled glucose analog 2-deoxy-D-glucose (FDG) [5], development of therapeutic strategies based on this metabolic shift still remains a challenge. Recent studies to unravel the intricate relationships between oncogenic transformation, enhanced glucose usage, degree of malignancy and therapeutic resistance of tumors [3,6] have reawakened research interest in the application of Warburg's hypothesis to identify and develop new therapeutic strategies.

A number of pharmacological agents that target glucose transport and/or specific glycolytic enzymes generally overexpressed in tumors are

currently under investigation in model systems and in clinical trials. Some promising agents include:

- 2-deoxy-D-glucose (2-DG), inhibitor of glucose transport, phosphohexoisomerase and glucose-6-phosphate dehydrogenase;
- 3-bromopyruvate and lonidamine, inhibitors of hexokinase;
- Inhibitors of pyruvate kinase subtype M2, found mainly in proliferating cells [7–9].

The glucose analog, 2-DG is the most widely investigated pharmacological agent in experimental and clinical oncology for targeting glucose metabolism. 2-DG competitively inhibits glucose transport (by sharing the same glucose transporters) and is phosphorylated by hexokinase (HK) to form 2-DG-6-phosphate, which is not metabolized further, thereby reducing the output from glycolysis (ATP) and the pentose-phosphate pathway (NAPDH) [10–12]. Furthermore, 2-DG alters N-linked glycosylation, leading to unfolded protein responses, and disrupts thiol metabolism, causing oxidative stress. It also induces changes in gene expression and the phosphorylation status of proteins involved in signaling, cell cycle control, DNA repair, calcium influx and cell death via apoptosis [13–15]. Inhibition of proliferation has been reported in many tumor cell lines, while animal studies have revealed a great deal of heterogeneity in the growth inhibition of tumors [16–19].

Initial clinical trials with intravenous infusions of 2-DG in leukemia and pancreatic tumor patients were discontinued due to adverse side effects, mainly in the form of diaphoresis and disturbances of the CNS caused by high doses

of 2-DG [20]. Recently, Phase I clinical trials in prostrate tumor patients have been initiated following encouraging results in preclinical studies [21]. While the results of these trials are still awaited, the potential of using 2-DG as a single therapeutic agent appears to be limited because of the likelihood of developing toxicity through daily administrations of large doses of 2-DG over a long period of time. The symptoms observed soon after single administration of pharmacological doses of 2-DG are similar to hypoglycemia, mainly due to glucocytopenia in the nervous system (hypothalamus), causing alterations in the regulation of cardio-respiratory and immune systems and other normal tissues [22]. Since 2-DG disappears from the blood with a half-life of approximately 90 min, the symptoms are transitory; however, continuous administration of 2-DG for a long duration could produce serious side effects.

“Design of treatment regimens based on the knowledge of time-dependent changes in the metabolic and cell signaling modifications caused by 2-DG *vis-à-vis* the time course of pro-survival and pro-death pathways is crucial for deriving optimal benefit from the combination.”

A more promising approach suggested by Jain, Pohlitz and Purohit, based on their studies on the energetics of repair of radiation damage in wild-type and respiratory-deficient mutants of yeast cells [23], is to use 2-DG in combination with anticancer agents as a differential modifier of repair processes. This could enhance the efficacies of currently popular therapeutic agents such as radiation and/or cytotoxic drugs [24]. Several *in vitro* and *in vivo* studies on various model systems have validated this approach by demonstrating that 2-DG administration before or immediately after irradiation can selectively enhance the induced killing of malignant cells by inhibiting energy-requiring cellular recovery processes involving DNA damage repair and cell cycle check points. The 2-DG-induced enhancement of radiation damage was observed to be directly proportional to the glucose usage, presence of hypoxia and doses of 2-DG and radiation [24–26]. It is noteworthy that in normal cell systems under similar conditions, reduction of damage (protection) has been observed [24,27]. While the precise molecular mechanisms underlying the 2-DG-induced differential modifications remain

yet to be elucidated, appropriate combinations of tumoricidal agents such as ionizing radiation and chemotherapeutic drugs with 2-DG provide unique opportunities to selectively destroy tumors, reduce toxicity to normal tissues and significantly enhance the therapeutic efficacy.

Based on the 2-DG-induced radiosensitization observed in primary organ cultures of cerebral glioma tissues [28], clinical trials were designed taking into consideration the radiobiology of gliomas and the pharmacokinetics of 2-DG. Since molecular and cellular processes related to survival and death of cells are initiated soon after irradiation and last from several minutes to a few hours (a few days in case of delayed metabolic oxidative stress), the design of treatment regimens based on the knowledge of time-dependent changes in the metabolic and cell signaling modifications caused by 2-DG *vis-à-vis* the time course of pro-survival and pro-death pathways is crucial for deriving optimal benefit from the combination. Preclinical studies using a combination of 2-DG administered intravenously just prior to (<5 min before) irradiation of the tumor showed excellent results, with tumor-free survival rates of 50–60% [24,29,30]. Therefore, the first clinical protocol to test the feasibility and safety consisted of an oral dose of 2-DG (200 mg/kg b.wt) given 20–30 min before γ - or photon irradiation, with four single weekly fractions of 5 Gy/fraction [31]. Subsequent dose escalation and multicenter Phase II trials on glioblastoma patients showed excellent compliance, tolerance and safety to the combined treatment of up to a seven-times weekly 2-DG dose of 250 mg/kg b.wt combined with a 5 Gy/fraction radiation dose [32]. At a higher dose of 300 mg/kg b.wt, two out of six patients could not complete treatment due to restlessness, although the vital parameters were not altered [32]. Enhanced survival seen at 2-DG doses of 250–300 mg/kg b.wt suggests that patients who tolerate higher doses would benefit the most from the combined treatment. Most importantly, these trials involving more than 100 glioblastoma patients indicated that the combination of 2-DG with hypofractionated radiotherapy significantly improved the quality of life, with clinicopathological evidence confirming the protection of normal brain tissue [33]. The treatment protocol using weekly large radiation fractions requiring administration of 2-DG only once a week, is convenient for the patient, cost-effective and permits treatment of a larger number of patients per machine. A Phase III multicentric trial is in progress.

Since radiosensitization by 2-DG increases with radiation dose, a clinical protocol for the treatment of glioblastoma with high-dose stereotactic radiosurgery was designed at the Kettering Medical Center (OH, USA) and approved by the US FDA. A clinical trial using a similar protocol to treat intracranial metastatic lesions is being carried out at the University of Iowa (IA, USA). Results of this trial should provide a basis for the treatment of other inoperable neoplasms with radiosurgery plus 2-DG.

The feasibility of using 2-DG in combination with nuclear medicine therapeutic regimens was demonstrated in metastatic thyroid carcinoma patients [24]. Protection of normal bone-marrow cells in whole-body irradiation [24,27] and sensitization of tumor cells to radionuclide-induced damage by 2-DG has been reported [34,35]. These preliminary investigations should encourage larger systematic studies to explore applications of 2-DG for improving the selectivity and efficacy of internal radionuclide therapy, particularly in metastatic diseases. However, optimal design of treatment and dose scheduling should be carefully chosen, as a reduction in the efficacy of radioimmunotherapy using 2-DG was recently reported in a preclinical study [36].

“Future clinical trials should be designed to study, noninvasively and quantitatively, correlations between glucose usage and 2-DG-induced effects in malignant and treatment-resistant tumors at various sites, as well as in normal tissues.”

Since cellular responses to damage caused by many anticancer drugs are similar to radiation, 2-DG is also being investigated as an adjuvant in cancer chemotherapy. Although *in vitro* studies have shown enhanced cell death [37,38], *in vivo* studies with different anticancer drugs and protocols have demonstrated limited success in obtaining local tumor control [39–40]. Although Phase I clinical trials have begun in the treatment of certain solid tumors [41], further studies are required to optimize treatment schedules for enhancing chemosensitization of tumors by 2-DG.

To identify patients who would most likely benefit from the combination of 2-DG and radiation or drugs, future clinical trials should be designed to study, noninvasively and quantitatively, correlations between glucose usage and 2-DG-induced effects in malignant and treatment-resistant tumors at various sites, as well as in normal tissues. Attempts should be made to further improve tolerance to higher doses of 2-DG, reduce toxicity and enhance the degree of tumor radiosensitization. Locoregional administration, dietary modifications, relaxation techniques (meditation) or use of sedatives such as diazepam that reduce glucose utilization in the brain could serve to enhance tolerance to 2-DG. Some other novel approaches that deserve to be tested in the clinic include: the use of low doses of 2-DG with the high fat/low carbohydrate ketogenic diet [42]; the combination of 2-DG with inhibitors of the pentose phosphate pathway, such as 6-aminonicotinamide [43,44]; and enhancing tumor glucose usage through the use of tumor-localizing agents such as hematoporphyrin derivatives [45] via intra-tumoral administration.

While research to elucidate precise mechanisms underlying the enhanced glucose metabolism in tumors and its relationship with treatment resistance should be intensified, the success of therapies targeting this phenotype with 2-DG will depend on reducing toxicity to vital organs by optimal design of protocols using novel approaches. For this to happen within a reasonable timespan, in order to make an impact on future oncology, biomedical scientists well-versed with the concepts and tools of molecular genetics, metabolic engineering and systems biology need to work in closer cooperation with clinical oncologists.

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