Enhancing Targeted Radiotherapy by Copper(II)diaacetly-bis(\(N^4\)-methylthiosemicarbazone) Using 2-Deoxy-D-Glucose

Rebecca L. Aft, 2 Jason S. Lewis, Fanjie Zhang, Joonyoung Kim, and Michael J. Welch


ABSTRACT

Most cancer deaths are a consequence of resistance to conventional chemotherapy and radiation therapy. This may be attributable to unique phenotypic characteristics of solid tumors. We have exploited two well-described characteristics of solid tumors commonly associated with treatment failure, high glucose use and hypoxia, to design a unique therapy based on the selective accumulation of two cytotoxic compounds, 2-deoxyglucose (2-DG) and copper(II)diaacetly-bis(\(N^4\)-methylthiosemicarbazone) (\(^{64}\)Cu-ATSM). \(^{64}\)Cu-ATSM localizes to hypoxic regions of tumors and has been used for administering a high local dose of radiation therapy after uptake by cells. 2-DG, a glucose analog, selectively accumulates in cancer cells and interferes with energy metabolism, resulting in cancer cell death. 2-DG has been shown to potentiate the cytotoxic effect of ionizing radiation and certain chemotherapeutic agents. We have tested the effect of 2-DG on tumor response when combined with \(^{64}\)Cu-ATSM in a mouse breast tumor model using the highly aggressive mouse mammary carcinoma cell line EMT-6. 2-DG administered up to 2 mg/g of body weight daily resulted in no weight loss or systemic symptoms. EMT-6 mammary tumors had similar uptake of \(^{64}\)F]fluoro-2-deoxyglucose before and after 2 weeks of 2-DG treatment as determined by microPET imaging, indicating that resistance to 2-DG uptake does not develop. Pretreatment of tumor-bearing mice with 2-DG resulted in increased uptake of \(^{64}\)Cu-ATSM by tumors compared with nontreated mice. This effect was not observed with the nonhypoxia-specific agent copper(II)pyruvaldehyde-bis(\(N^4\)-methylthiosemicarbazone). When 2-DG was combined with a single dose of \(^{64}\)Cu-ATSM (2 mCi), tumor growth was inhibited ~60% compared with untreated mice, and animals survived ~50% longer than untreated mice or animals treated with each agent alone (32 versus 20 days). The maximum effect on tumor growth and survival was observed when 2-DG was administered daily for the lifetime of the mouse. Our results indicate that 2-DG potentiates the effect of \(^{64}\)Cu-ATSM on tumoricidal activity and animal survival. We hypothesize that 2-DG alters the metabolic state of the cell, leading to increased uptake of \(^{64}\)Cu-ATSM by the tumor. This would result in a higher local dose of radiotherapy. The continued presence of 2-DG would then prevent the repair of damaged cells, leading to inhibition of tumor growth. Our data indicate that the strategy of combining tumor-specific cytotoxic agents that function by differing mechanisms can result in an effective, selective, tumor-specific cell death with minimal effect on the host.

INTRODUCTION

Breast cancer is the most common malignancy diagnosed in American women and is currently the second most frequent cause of cancer death despite early detection and treatment. Progression of disease is the result of treatment failure, which is a consequence of resistance to conventional therapy. Resistance to chemotherapy and radiation therapy may be attributable to phenotypic characteristics of the cancer cells (1). Multiple strategies have been implemented with varying effectiveness to overcome treatment-refractory cancers including combination chemotherapy with agents differing in mechanism, sensitizers to radiation therapy, and inhibitors of receptors (2–5). In this series of experiments, we have tested the efficacy of combining two tumor-specific cytotoxic agents in a mouse model of breast cancer to develop a targeted therapy for breast tumors.

Human tumors preferentially use glycolysis for energy production, even under aerobic conditions (Warburg effect), although this requires more glucose to generate energy and support synthetic functions (6, 7). Glucose use may represent an important regulatory point in the maintenance of the growth and synthetic activity of malignant cells and suppression of apoptosis (8, 9). Our previous experiments confirm this finding and further demonstrate that functional glucose deprivation achieved using the nonmetabolizable glucose analog 2-DG causes apoptosis in breast cancer cells (10). 2-DG is a structural analog of glucose that selectively accumulates in cancer cells after phosphorylation by hexokinase (Fig. 1A). Selective accumulation of 2-DG has been attributed to several metabolic alterations observed in cancer cells including increased glucose transporter expression, resulting in increased uptake (11–13), increased hexokinase activity, and low phosphorylase activity, resulting in intracellular trapping (14–18). Inhibition of glycolysis by phosphorylated 2-DG, 2-DG-6-phosphate, is thought to occur via inhibition of hexokinase and phosphohexoisomerase (19, 20). Cancer cell death by 2-DG may be through intracellular glucose deprivation, resulting in induction of stress-related proteins (21, 22), the generation of free radicals (23), or inhibition of energy metabolism (22–24). Tumor-bearing animals treated with 2-DG exhibit impairment of local tumor growth (18, 25). Recent clinical trials administering 2-DG in combination with IR to treat brain gliomas indicate that oral 2-DG is well tolerated by patients and may be protective to normal surrounding tissues (26).

\(^{64}\)Cu-ATSM has been used as an imaging agent to delineate hypoxic areas within tumors (27–31) and as an agent for targeted radiotherapy (32, 33). \(^{64}\)Cu-ATSM is a neutral lipophilic molecule of low molecular weight, high membrane permeability, and low redox potential (Fig. 1B; Ref. 27). It is thought that bioreduction of the \(^{64}\)Cu(II)-ATSM complex intracellularly leads to the release of \(^{64}\)Cu(I), which then becomes bound to intracellular macromolecules. This process occurs more readily in hypoxic tissue compared with normoxic tissue (28, 34, 35). Uptake of \(^{64}\)Cu-ATSM by tumors in cervical cancer patients correlates with patient survival and is similar to results obtained using oxygen electrodes to discriminate hypoxic versus nonhypoxic cervical cancers, supporting the use of \(^{64}\)Cu-ATSM PET imaging as a method for the clinical examination of tumor hypoxia (30). Similar results have been reported recently in patients with lung cancer (31). \(^{64}\)Cu linked to small, tumor-specific molecules...
has been used as an agent for administering high-dose local radiotherapy. Experiments using $^{64}$Cu linked to monoclonal antibodies, ATSM, or PTSM have demonstrated that $^{64}$Cu can inhibit solid tumor growth and prevent peritoneal spread of cancer cells (32, 36–38). During decay, $^{64}$Cu emits a 0.58-MeV $\beta$-particle (40%), a 0.66-MeV $\beta^+$ particle (19%), and a y of 1.34 MeV (0.5%), giving a wide variety of potentially radiotoxic emissions. Moreover, during decay by electron capture, the copper radionuclide emits highly radiotoxic Auger electrons with high linear energy transfer, which have a tissue penetration of 0.02–10 $\mu$m. Because of the short range and relatively larger linear energy transfer, Auger electrons are potentially more radiotoxic than the higher energy $\beta$-particles if localized in the nucleus (39, 40).

Hypoxic tumors are relatively resistant to conventional therapy, and hypoxia is associated with a more aggressive malignant phenotype (41). Tumor hypoxia appears to be a dynamic process dependent on blood flow and tumor vasculature, resulting in areas of acute and chronically hypoxic cells (1). Increased expression of the glucose transporter has been found in areas of relative hypoxia in solid tumors (17). We and others have demonstrated that 2-DG is an effective sensitizer in causing cancer cell death (42, 43). From these experimental findings, we propose that 2-DG treatment of cancer cells causes apoptosis (10). Transformed cells appear to be more sensitive to the effect of 2-DG than their normal counterparts (42).

When combined with IR or certain chemotherapeutic agents, 2-DG acts as a sensitizer in causing cancer cell death. From these experiments, we predicted that 2-DG would enhance the effect of other tumor-specific therapies.
2-DG has been administered p.o., i.p., and i.v. in several animal tumor models (18, 50, 51). We first determined the optimal method of administering 2-DG using [18F]FDG as a tracer and surrogate for 2-DG. This allowed the use of microPET imaging to examine organ distribution. Although the affinity for enzymes involved in the metabolism of 2-DG and FDG may differ, both FDG and 2-DG enter cells through the glucose transporter and are both phosphorylated by hexokinase (52). 2-DG was mixed with [18F]FDG and administered i.g., i.v., i.p., or s.c. to mice. Distribution of the [18F]FDG was determined at 1 and 3 h after administration. As seen in Fig. 2A, the majority of the [18F]FDG remained in the stomach at 3 h when administered i.g. When 2-DG/[18F]FDG was injected s.c. in the right flank, the majority of the radioactivity was retained in the s.c. tissue over the right kidney at 1 h, and by 3 h most of the [18F]FDG had been absorbed (Fig. 2B). 2-DG/[18F]FDG injected either i.v. or i.p. resulted in a similar distribution between organs and comparable SUVs at 1 and 3 h (Fig. 2, C and D). The majority of the tracer at 1 h was detected in the brain, heart, and kidneys. The SUVs for these organs decreased ~50% by 3 h. These results indicate that [18F]FDG mixed with 2-DG is readily absorbed when given i.p. or i.v. In all subsequent treatment experiments, 2-DG was administered i.p.

We next determined the optimal dose of 2-DG. Previous experiments have reported the LD50 for 2-DG as 4.5 mg/g of body weight when injected i.p. in mice (25), and in humans doses up to 200 mg/kg i.v. or p.o. have been administered (50, 53). 2-DG was injected i.p. daily at doses from 0.5 mg/g of body weight to 2 mg/g of body weight. Doses up to 2 mg/g of body weight (40 mg) injected i.p. daily resulted in no weight loss, change in appetite, or other constitutional symptoms (Fig. 3A).

To determine the effect of 2-DG on tumor growth and uptake of [18F]FDG, we used the highly aggressive EMT-6 mouse mammary carcinoma cell line as a breast tumor model. This cell line has been well characterized and used extensively to investigate hypoxic agents because EMT-6 solid tumors contain a significant hypoxic fraction dependent on the size of the tumor (54–56). Untreated EMT-6 tumors will double in volume every 4–5 days. 2-DG treatment beginning day 4 after tumor implantation resulted in a mild inhibition of tumor growth (Fig. 3B). For example on day 13, 2-DG given at 40 mg/mouse inhibited tumor growth by ~40%. No systemic toxicity was observed at this dose. Higher doses of 2-DG in non-tumor-bearing mice led to postinjection lethargy. Overall survival was unchanged at all doses of 2-DG treatment compared with control mice.

EMT-6 tumors established in BALB/c mice selectively accumulate FDG as determined by [18F]FDG microPET imaging (Fig. 4). MicroPET imaging was performed using [18F]FDG on various days after commencing daily treatment with 2-DG to determine whether uptake changed over time of treatment. It has been reported that long-term growth of cells in vitro in the presence of 2-DG can result in decreased
2-DG-induced toxicity, thought to be related to changes in the transport activity of Glut 1 (57). Mice bearing EMT-6 tumors were treated with 2-DG daily at 40 mg/mouse (2 mg/g) and imaged on days 1, 7, 9, 11, and 14 after the start of treatment. On the day of imaging, 2-DG was coinjected with [18 F]FDG in animals undergoing treatment. Brain was used as a control because it has high uptake of [18 F]FDG. As seen in Fig. 5A, 18 F-FDG uptake by brain was relatively constant for the duration of the treatment with and without 2-DG treatment. The amount of uptake of [18 F]FDG by the brain at all time points was slightly less than half of nontreated animals. Untreated EMT-6 tumors increased the amount of [18 F]FDG uptake to day 7, and then this gradually decreased (Fig. 5B, left panel). EMT-6 tumors treated with 2-DG maintained a relatively constant uptake of [18 F]FDG throughout the treatment period, which was lower than the amount taken up by nontreated animals (Fig. 5B, right panel). These data indicate that tumor resistance to uptake of [18 F]FDG did not develop during the 2 weeks of treatment with 2-DG, although the constant presence of 2-DG may have affected the tumor cell biology because the peak at 7 days was not observed. These data demonstrate that EMT-6 tumors continue to accumulate [18 F]FDG throughout the treatment interval with 2-DG. These data would suggest that resistance to 2-DG uptake by tumor cells does not develop during this time interval of treatment.

The effect of 2-DG on tumor uptake of 64 Cu-ATSM and 64 Cu-PTSM was next determined using microPET imaging and by calculating time-activity curves. 64 Cu-ATSM and 64 Cu-PTSM are compounds of low molecular weight and high membrane permeability that differ by a methyl group in the bis(thiosemicarbazone) complex (Fig. 1B). This change causes a marked difference in biological properties (35). 64 Cu-PTSM rapidly accumulates in tissues with high blood flow and has been used as a flow indicator (58). With bolus injection, it is retained in tissues by first-pass extraction (59). In contrast to 64 Cu-ATSM, which has been used to delineate hypoxic tissues (27, 28, 34). In vivo, 64 Cu-ATSM is rapidly taken up by all tissues; however, it is washed out of nornoxic tissues over time, and the copper remains only in hypoxic cells (28, 33). The difference in properties between 64 Cu-ATSM and 64 Cu-PTSM are thought to be attributable to the redox potentials of the complexes (28). The redox potential of 64 Cu-PTSM is −208 mV, and 64 Cu-ATSM is −297 mV. 64 Cu-PTSM is reduced in normal tissues at the complex I site of the electron transport chain using NADH as the electron donor (60). Under normoxic conditions, 64 Cu-ATSM is reduced less efficiently than 64 Cu-PTSM at the complex I site. It is thought that under hypoxic conditions, depletion of oxygen causes hyperreduction of complex I and an increase in NADH concentration, which is thought to account for the increased retention of 64 Cu from 64 Cu-ATSM in hypoxic tissues (35). Alternatively, 64 Cu retention from 64 Cu-ATSM in cancer cells may be attributable to reduction of the copper by the microsomal fraction of tumor cells rather than by mitochondria-associated enzymes (61). In PET imaging of 64 Cu-ATSM or 64 Cu-PTSM, it is the 64 Cu radioactive decay and its localization that is collated, regardless of the chemical and oxidation state of the copper.

2-DG was administered to mice 1 day before and on the day of the imaging. The brain was used as a control organ for the comparison between 64 Cu-ATSM and 64 Cu-PTSM. As seen in Fig. 6A, 64 Cu-PTSM rapidly crosses the blood-brain barrier and is retained in the brain tissue. 64 Cu-ATSM, although initially extracted efficiently from
the blood pool into the brain, is not reduced intracellularly and is released from the cells (Fig. 6C). The $^{64}$Cu-PTSM values are lower than $^{64}$Cu-ATSM because there has been uptake of $^{64}$Cu-PTSM in other organs before brain delivery, and thus, less activity is available for extraction. $^{64}$Cu-ATSM is released from normoxic tissue, and therefore, more is delivered to the brain. As evidenced by the time-activity curves, $^{64}$Cu-ATSM is subsequently washed out over time. This is consistent with results reported previously (28). EMT-6 tumors demonstrated a rapid uptake and retention of $^{64}$Cu-PTSM, similar to that observed with brain, in both control and 2-DG-treated animals, indicating a high blood flow to the tumor (Fig. 6B). In contrast, when $^{64}$Cu-ATSM was used as the tracer, an increase in uptake and retention within tumor was observed with 2-DG treatment that was not observed in control animals (Fig. 6D). Uptake and retention of $^{64}$Cu-ATSM by EMT-6 tumors was increased up to 5-fold with 40 mg/mouse 2-DG treatment (Fig. 6D), whereas 10 mg/mouse 2-DG caused a 2-fold increased uptake and retention of 2-DG. The 2-DG effect on uptake and retention was only observed for the hypoxia-specific agent $^{64}$Cu-ATSM and not with the nonhypoxia-selective agent $^{64}$Cu-PTSM. These data indicate that 2-DG pretreatment of animals leads to an increased uptake and retention of the hypoxia-specific agent $^{64}$Cu-ATSM by tumors.

Given that 2-DG treatment of EMT-6 tumor-bearing mice leads to the increased accumulation of $^{64}$Cu-ATSM within tumors, we hypothesized that this combined treatment would lead to a higher local dose of radiation therapy, resulting in a more efficient tumor cell killing. This would translate into greater inhibition of tumor growth and improved overall survival of the animals. To test this, EMT-6 tumor-bearing mice were injected with 2-DG daily and given a single dose (2 mCi) of either $^{64}$Cu-ATSM or $^{64}$Cu-PTSM. Combining 2-DG with either $^{64}$Cu-ATSM or $^{64}$Cu-PTSM caused a relative inhibition of tumor growth (Fig. 7). Mice treated with $^{64}$Cu-PTSM plus 2-DG...
DEOXYGLUCOSE-ENHANCED TARGETED THERAPY WITH ⁶⁴⁸CU-ATSM

**DISCUSSION**

Alterations in the activity of processes that affect cellular metabolism may affect the sensitivity of cancer cells to other therapies. This has been the basis of combination chemotherapy and radiation therapies. We have shown previously that 2-DG-induced metabolic alterations cause apoptosis of cancer cells and significantly potentiate the cytotoxic effects of ionizing radiation in human cancer cell lines (10, 42). In the present study, we demonstrate that treatment with 2-DG potentiates the cytotoxic effect of targeted radiation therapy with ⁶⁴⁸Cu-ATSM in a mouse tumor model of aggressive breast cancer.

The efficacy of conventional radiotherapy is limited by the presence of a hypoxic, radioresistant, and repair-proficient subset of tumor cells. This has led to the development and testing of radiosensitizers. Previously tested radiosensitizers have been limited by their clinical toxicity and lack of clinical effectiveness (1, 3). In the present study, we have used a hypoxic tumor model to demonstrate that 2-DG acts as a radiosensitizer for ⁶⁴⁸Cu-ATSM. ⁶⁴⁸Cu-ATSM is selectively retained in hypoxic cells in vitro and tumor cells in vivo (28) and has been used for targeted radiotherapy in animal models of tumors and tumor metastases (28, 34). In patients with cervical carcinoma, accu-

![Image](5501)

**Fig. 7.** Effect of 2-DG on tumor growth after treatment with ⁶⁴⁸Cu-ATSM or ⁶⁴⁸Cu-PTSM. EMT-6 tumors were implanted on day zero. Mice received daily i.p. injections with 2-DG (40 mg/mouse) beginning on day 4. A single dose (2 mCi/mouse) of either ⁶⁴⁸Cu-PTSM (A) or ⁶⁴⁸Cu-ATSM (B) was injected i.v. on day 5 where indicated. Tumor size and animal weights were recorded daily. Mice were sacrificed if the tumor ulcerated or was >10% of body weight. Each point represents the average tumor volume or weight of five mice except for the treatment groups ⁶⁴⁸Cu-PTSM alone and with 2-DG. These two groups contained four mice only because of the nongrowth of tumors in two mice. Error bars represent the range of tumor size. A, mice treated with ⁶⁴⁸Cu-PTSM with or without 2-DG. B, mice treated with ⁶⁴⁸Cu-ATSM with or without 2-DG.

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**Fig. 8.** Effect of 2-DG on survival after treatment with ⁶⁴⁸Cu-ATSM or ⁶⁴⁸Cu-PTSM. Estimated survival distribution function was generated using a Kaplan-Meier time-to-death analysis. For comparison involving treatments with ⁶⁴⁸Cu-PTSM treatments with or without 2-DG, the log-rank test gives a $P$ of 0.29, indicating that there are no significant differences between groups (A). The ⁶⁴⁸Cu-ATSM treatments with or without 2-DG comparison has a $P$ of 0.011 from the same test, indicating that there are statistically significant differences between the groups (B).

when 2-DG was administered for 5 days compared with controls. The maximal effect was observed when 2-DG was present for the lifetime of the mouse.

The $t_{1/2}$ of ⁶⁴⁸Cu is 12 h, and ⁶⁴⁸Cu is completely decayed by 5 days. To determine whether 2-DG needed to be present during the peak activity of ⁶⁴⁸Cu or the lifetime of the mouse, 2-DG was given for a total of 6 days (1 day before treatment and 5 days after treatment with ⁶⁴⁸Cu-ATSM). As seen in Fig. 8B, there was no change in survival
mulation of $^{64}$Cu-ATSM correlated with tumor hypoxia and poor patient outcome (30).

Copper radioisotopes have been studied extensively for therapeutic use (62, 63). $^{64}$Cu has been identified as an attractive radionuclide for therapy because it decays with a number of therapeutic emissions, has a short half-life (12.7 h), is a natural constituent of DNA, and functions as a copper cation in cells (63). Furthermore, $^{64}$Cu decays with $\beta^+$ emissions, which allows for simultaneous imaging by PET (64, 65). The radiobiological effect of $^{64}$Cu is complex because of the multiple types of emissions, and cell kill may depend on the intracellular distribution of the radionuclide. Previous studies have shown that $^{64}$Cu is cytotoxic, as shown by a linear cell survival curve when plotted on a logarithmic scale (62, 63). This is an interesting result, considering that $^{64}$Cu is considered a low LET nuclide, and the survival curves of low LET nuclides generally have a large shoulder. Low LET decay is characteristic of $\beta^+$-emitters, and high LET decay is characteristic of nuclides that decay by particles or Auger electrons (66–68). With radionuclides such as $^{64}$Cu, which have both $\beta$ and Auger emissions, the copper ion needs to be incorporated in the cell nucleus for the Auger electrons to cause cell kill because of the short path length of emitted particles. This is in contrast to the $\beta$ emission, which can result in a “cross-fire” energy from neighboring cells. Because copper has an important role in the function of the cell nucleus, playing a structural role in nuclear matrix proteins, copper trafficking to the cell nucleus may partially explain the behavior of $^{64}$Cu as a high LET emitter. If the copper radionuclide were in the vicinity of the DNA upon decay, the daughter species could collide (“recoil”) with the DNA, causing irreparable damage and cell death. Multiple factors require consideration to understand the relative biological effectiveness of the emitted radiations of $^{64}$Cu, including the time-dose pattern, the distribution of the radionuclide in the target tissue within the cell, and the absorbed dose/energy. Unfortunately, little data are available concerning the influence of this latter factor. It is clear that there is an increasing need to understand the complexities of the dose rate in radiotherapy studies, which would allow a more exacting determination of biological response to radiotherapy.

High glucose transporter levels have been found in areas of tumor hypoxia and in cancer cells that are resistant to cytotoxic drugs and ionizing radiation (17, 20, 69). It has been suggested that high glucose transporter expression is a survival response of cancer cells that allows growth in poor nutrient conditions and counteracts apoptotic signals in cancer cells (8, 9). $^{64}$Cu accumulates in cells by facilitated diffusion through the glucose transporter (11, 17). Because of intrinsic metabolic alterations in cancer cells, $^{64}$Cu becomes selectively trapped within cancer cells (14). Furthermore, hypoxic conditions lead to an increase in $^{64}$Cu uptake in vitro (17, 69, 71). These data suggest that $^{64}$Cu localizes to cancer cells, which may be particularly resistant to chemotherapy or radiation therapy.

Daily injections of $^{64}$Cu do not appear to alter the uptake of $[^{18}\text{F}]\text{FDG}$ accumulation at various times after initiation of treatment. This indicates that tumors do not become resistant to $^{64}$Cu uptake over time, as has been reported in tissue culture (57). Untreated EMT-6 tumors gradually increase $[^{18}\text{F}]\text{FDG}$ uptake to a peak at 7 days. This may reflect the evolving biology of tumor growth, which leads to increasing hypoxia followed by angiogenesis (1) or a change in the mass of viable cells. We have reported previously that $^{64}$Cu treatment of breast cancer cells in vitro results in an increased expression of glucose transporter and an increase in glucose uptake (10). It is possible that a similar phenomenon occurs in solid tumors. Tumor cells may adapt to the presence of $^{64}$Cu by altering transporter levels such that they can maintain an adequate level of glucose uptake because there may be a limited ability to adapt to other forms of energy metabolism.

Pretreatment with 2-DG results in increased uptake and retention of $^{64}$Cu-ATSM by EMT-6 mouse breast cancer tumors. In vitro, treatment with 2-DG causes rapid generation of reactive oxygen species and alterations in intracellular thiol levels (23, 24, 42). These intracellular alterations may account for the increased accumulation of $^{64}$Cu-ATSM within the breast cancer tumors after 2-DG treatment. The observed increased accumulation of $^{64}$Cu-ATSM may be a result of greater uptake by each individual tumor cell or by uptake by a greater percentage of cells. Experiments comparing the distribution of $[^{18}\text{F}]\text{FDG}$ and $^{64}$Cu-ATSM in solid tumors revealed regions of $^{64}$Cu-ATSM uptake that were distinctly different from regions of increased uptake of $[^{18}\text{F}]\text{FDG}$, indicating that $[^{18}\text{F}]\text{FDG}$ and $^{64}$Cu-ATSM localize to different regions of the tumor (33). In the tumor microenvironment, cells in close proximity to the vasculature would presumably have higher $[^{18}\text{F}]\text{FDG}$ uptake compared with cells remote from the vascular bed, despite the higher glucose transporter levels found in hypoxic tissue attributable to the increased availability. It is therefore tempting to speculate that those tumor cells with high $[^{18}\text{F}]\text{FDG}$ uptake that are not hypoxic or are chronically hypoxic may be altered metabolically by 2-DG such that $^{64}$Cu-ATSM accumulates in these cells. This may result in a more homogeneous distribution of $^{64}$Cu-ATSM within the tumor, which could result in a greater tumor cell kill. Toxic Auger particle emissions have a mean range of penetrating radiation only in the nanometer range (40), more cells would be affected, and the contribution of cross-fire to cell kill from the $\beta^+$ emission would increase. Maximal tumoricidal effect would therefore be anticipated with a more uniform distribution of $^{64}$Cu throughout the tumor. Alternatively, the administration of 2-DG may result in an increase in uptake of $^{64}$Cu-ATSM into the hypoxic regions. Consequently, the Auger emission would be even more effective within the hypoxic region before addition of the radiosensitizer. After the addition of the radiosensitizer, all of the longer ranged $\beta^+$ emissions could be providing the enhanced therapy to the normoxic areas of the tumor. The mechanism underlying the effect of 2-DG on $^{64}$Cu-ATSM accumulation is currently under investigation in our laboratories.

The maximal effect of 2-DG was observed when the therapy was administered for the duration of the animal’s life. In vivo studies in animal tumors have shown that administration of 2-DG just before irradiation results in a significant delay in tumor growth, leading to an improvement in survival of animals (43). Although 2-DG alone causes cancer cells to undergo apoptosis in vitro (10, 23), it is likely that the effects of 2-DG in enhancing radiation sensitivity are multifactorial and include effects on the metabolic state of the cell, resulting in a depletion of radioprotective sulfhydryl groups (42) and depletion of ATP, which in turn results in the inability of the cell to repair radiation-induced damage (72). A substantial body of data suggests that DNA is the target of the cytotoxic effects of radiation (73). Double-strand DNA breaks are responsible for radiation-induced cellular death, and differences in cellular sensitivity to radiation may reflect the ability of the cell to repair this damage. 2-DG treatment through depletion of intracellular energy may impair the ability of the cell to repair double-stranded DNA breaks, and as a consequence, the number of lethal lesions increases (74). Gene activation, transcription, and protein synthesis in response to radiation damage are required for the cell to survive a dose of radiation. These cellular processes leading to error-free repair require continuous metabolic energy, which is frequently supplied by enhanced glycolysis. The presence of 2-DG for several hours after radiation has been reported to selectively inhibit the postirradiation repair processes in cells, thereby enhancing radiation damage (75). Finally, 2-DG by changing the metabolic state of the cell may lead to depletion in the cell of radioprotective thiols, which may act as free radical scavengers (42).

For targeted therapy to be useful, there must be greater delivery of
the cytotoxic agent to tumor cells than to normal cells (76). In these experiments, we have used 2-DG to enhance the cytotoxic effect of 64Cu-ATSM. Each of these agents is selectively accumulated to higher concentration in tumors compared with normal tissues as a result of physiological differences between tumors and normal cells. In general, using two cancer cell-selective agents should increase the toxicity to cancer cells while minimizing damage to normal tissue. Furthermore, disseminated tumors cannot be targeted with conventional radiation. Biologically targeted radiotherapy with agents such as 44Cu-ATSM are designed to overcome this constraint and would theoretically allow treatment of metastatic disease (76). A limitation of targeted radiotherapy has been the heterogeneity of radionuclide uptake on a cell-to-cell basis and within a tumor, which may limit clinical effectiveness of these forms of treatment. One approach to overcome this limitation may be to use a combination of tumor-specific cytotoxic agents or radiosensitizers.

The results of this study are of potential clinical interest and may provide a rationale for combining agents such as 2-DG and 64Cu-ATSM in the treatment of relatively hypoxic human tumors with high glucose uptake. Pretreatment imaging with [18F]FDG and 64Cu-ATSM would allow stratification of tumor phenotypes such that those tumors that are most susceptible would be selected for treatment. Although 64Cu-ATSM has not yet been tested as a therapeutic agent in human studies, the absorbed dose estimates for 64Cu-ATSM have been calculated by extrapolation from a hamster model (32). In this report, an estimated dose of 7500 mCi of 64Cu-ATSM for clinical trials in humans would be possible. Absorbed doses are currently being calculated at Washington University in humans using 64Cu-ATSM and PET in conjunction with the recently reported papers on 60Cu-ATSM in cervical and non-small cell lung cancer patients (30, 31). 2-DG at doses up to 200 mg/kg has been used in Phase I trials (53). It is possible that clinically effective therapeutic levels may be achievable using this combination of drugs rather than each individually. This type of combination may provide a unique treatment for chemotherapy-refractory tumors as well as disseminated disease. Although the current studies focus on breast cancer, hypoxia and increased glucose transport are observed in a wide variety of primary solid tumors and metastatic disease. We think that combinations such as 2-DG and 64Cu-ATSM will result in an effective, selective treatment for solid tumors and provide a new therapy for control of local and distant disease.

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