Colorectal Tumor Cells Treated with 5-FU, Oxaliplatin, Irinotecan, and Cetuximab Exhibit Changes in ^{18}F-FDG Incorporation Corresponding to Hexokinase Activity and Glucose Transport

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The purpose of this study was to determine therapy-induced changes in ^{18}F-FDG incorporation at the colorectal tumor cell level in response to conventional and novel chemotherapy agents and examine how these changes relate to factors involved in ^{18}F-FDG incorporation. **Methods:** SW620 cells were treated with inhibitory concentration of 50% (IC_{50}) doses (determined by MTT) of 5-fluorouracil (5-FU), oxaliplatin, and irinotecan; HCT-8 cells were treated with IC_{50} doses of irinotecan, cetuximab, and irinotecan plus cetuximab. ^{18}F-FDG incorporation, glucose transport, hexokinase (HK) activity, adenosine triphosphate (ATP) content, annexin V binding, and cell cycle distribution were determined after 24-, 48-, and 72-h treatments. Eight-hour treatments with and without subsequent incubation in drug-free medium were also examined. A clonogenic assay was used to determine the tumor-forming ability of treated cells. **Results:** Apoptosis was evident in SW620 cells, especially after treatment with irinotecan and 5-FU. ^{18}F-FDG incorporation was increased in SW620 cells after 24- or 48-h treatments with some agents and in HCT-8 cells after irinotecan treatment but was decreased in all 72-h treatments or cell-line combinations including cetuximab. Treatment of SW620 cells for 8 h followed by 64 h in drug-free medium also resulted in decreased ^{18}F-FDG incorporation. Decreased ^{18}F-FDG incorporation broadly corresponded to glucose transport in HCT-8 cells and to HK activity in SW620 cells. Inhibition of glucose transport decreased ^{18}F-FDG incorporation into HCT-8 but not into SW620 cells. ATP levels were decreased by oxaliplatin treatment and increased at 48 or 72 h after irinotecan treatment. **Conclusion:** ^{18}F-FDG incorporation is modulated by therapy-induced changes in both glucose transport and HK activity depending on the tumor cell. Colorectal cells treated with IC_{50} doses of cetuximab also exhibit decreased ^{18}F-FDG.

Key Words: oncology; PET; cetuximab; colorectal; ^{18}F-FDG; glucose transport

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Colorectal cancer (CRC) is the second most common cause of cancer death in the United States, with adenocarcinoma of the colon affecting about 5% of the population. Systemic chemotherapy doubles the survival of patients with advanced CRC. 5-Fluorouracil (5-FU) has been used with beneficial results for the last 40 y (*1*), and advances in the development of anticancer drugs during the past 10 y have resulted in improved patient outcomes. These include the use of the topoisomerase I inhibitor irinotecan, which resulted in improved response of patients who were refractory to 5-FU (*2*) and the platinum drug, oxaliplatin-based chemotherapy (*3*). However, oxaliplatin treatment is limited by neurotoxic side effects (*4*).

More recently, the use of several biologic therapies (e.g., cetuximab) has also improved survival in patients with metastatic CRC. Epidermal growth factor receptor (EGFR) is overexpressed in 70%–80% of CRC cancers and is targeted with antibodies such as the anti-EGFR monoclonal antibody cetuximab. Cetuximab, either alone or with irinotecan, has shown promising results in patients with metastatic CRC. Further improvement is brought about by the combined use of cetuximab and irinotecan, resulting in a higher response rate and longer time to progression as a first-line treatment of CRC (*5*).

The favorable option for large unresectable metastasis is downstaging to resectability (*6*); it is crucial to determine the combination of agents that will induce regression in these patients. Finding the combination of agents is facilitated by early detection of therapy response in patient management to avoid unnecessary drug exposure, in the case of nonresponders, and consequent side effects. A few studies (*7–11*) have determined ^{18}F-FDG incorporation by metastatic CRC responding to treatment with 5-FU, oxaliplatin, and irinotecan and showed, compared with pretreatment, decreased tumor ^{18}F-FDG incorporation during response. However, to our knowledge, no studies have determined the effect of cetuximab on ^{18}F-FDG incorporation by colorectal tumors.

Although ^{18}F-FDG incorporation is generally shown to be decreased by primary and secondary colorectal tumors

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during or after response, compared with pretreatment, what happens at the tumor cell level during response and the mechanism involved are poorly understood.

At the tumor cell level, ^{18}F-FDG incorporation has been shown to be dependent on glucose transport (*12*), hexokinase (HK) activity (*13*), and adenosine triphosphate (ATP) content (*14*). To understand how therapy-induced modulation of these factors contributes to ^{18}F-FDG incorporation and the potential of ^{18}F-FDG to monitor response to cetuximab as a monotherapy and combined with irinotecan, we have determined ^{18}F-FDG incorporation, glucose transport, HK activity, ATP content, and apoptosis in 2 CRC adenocarcinoma cell lines: SW620 cells treated with inhibitory concentration of 50% (IC_{50}) doses of 5-FU, irinotecan, and oxaliplatin and HCT-8 cells (which respond to cetuximab) treated with irinotecan, cetuximab, and a combination of the 2 therapies.

MATERIALS AND METHODS

Cells

SW620 and HCT-8 cells were purchased from the European Collection of Cell Cultures. Expression of EGFR by SW620 cells was considered negative, whereas expression of EGFR by HCT-8 cells was moderate (*15*). SW620 cells were maintained in Dulbecco's modified Eagle's medium (Invitrogen) supplemented with 10% fetal calf serum, 50 units of penicillin per milliliter, and 50 μg of streptomycin per milliliter. HCT-8 cells were incubated in RPMI 1640 medium supplemented with 5% horse serum, 5% fetal calf serum, 50 units of penicillin per milliliter, and 50 μg of streptomycin per milliliter.

MTT Assay

Cells with known seeding density in 100 μL of culture medium were plated onto 96-well plates with a surface area of 0.35 cm^3 and incubated at 37°C in a humidified atmosphere containing 5% carbon dioxide to allow the cells to attach to the wells. A control of cells in medium and background of medium alone was also plated. Twenty-four hours later, cells were exposed to varying concentrations of the drug (200 μL/well in total) and incubated at 37°C for 72 h. After exposure to the drug, the cells were treated with 50 μL of 5-mg MTT (3-(4,5-demethylthiazol-2-yl)-2,5-diphenyl tetrazonium bromide) dye per milliliter (Sigma; Dorset) dissolved in phosphate-buffered saline (PBS) and incubated for a further 4 h at 37°C. All liquid was then removed with a needle and syringe, and 200 μL of dimethyl sulfoxide was added to each well to dissolve the MTT-formazan crystals.

The plates were positioned in a scanning multiwell spectrophotometer (MR5000; Dynatech Laboratories Inc.). After shaking for 30 s, each plate was then photometrically quantified at an absorbance of 570 nm (test filter, 570 nm, and reference filter, 690 nm). All experiments were repeated 3 times with 6 replicates per experiment. The amount of purple formazan produced by treated cells was compared with that of the untreated control cells, and the absorbance in the treated cells was expressed as a percentage of control.

Colony-Forming Ability

The ability of cells treated for 8 and 72 h with IC_{50} doses of drug to proliferate was determined by seeding cells into 25-cm^2 tissue culture flasks (20,000/flask in triplicate per treatment) and finding the number of cells that formed colonies of at least 50 SW620 cells or 250 HCT-8 cells 6 d after treatment (results are expressed relative to number of cells that formed colonies in the control flasks) (*16*). Colony counts were performed using an inverting microscope (Eclipse TS100; Nikon) with a ×10 objective lens (individual cells could be clearly seen using this lens), counting cells in 20 fields per flask.

^{18}F-FDG Uptake

Uptake of ^{18}F-FDG was determined in cells untreated and cells exposed to the IC_{50} concentration of each drug determined from the MTT assay.

Cells cultured in 75-cm^3 tissue culture flasks approaching 80%−90% coverage were trypsinized and seeded into 25-cm^2 flasks (1 mL of cells, 4 mL of medium). Flasks with cells to be treated contained twice the number of cells of the control flasks (a 50% reduction of cell number provided a model of cells responding to treatment). After 24 h of incubation, medium was replaced with 10 mL of fresh medium, with or without the drug. Flasks were incubated for 24, 48, or 72 h, after which medium in each flask was replaced with 1 mL of fresh medium containing ^{18}F-FDG (37 kBq/mL) (John Mallard PET Centre) for 20 min. ^{18}F-FDG incorporation was then determined as described previously (*17*) and expressed relative to total protein. Protein content was determined using a bicinchoninic acid protein assay kit (Sigma).

Glucose Uptake

Glucose transport into cells was determined by evaluating the initial uptake of the glucose analog 3-*O*-methyl-D-[1-^{3}H]glucose (^{3}H-OMG) (Amersham Biosciences), a slowly metabolized glucose transporter substrate. The initial phase of glucose uptake by untreated cells was determined by incubating the cells in ^{3}H-OMG for the following times: 3, 5, 15, 20, 60, and 300 s. A midpoint in the initial uptake region (3 s for HCT-8 cells and 5 s for SW620 cells) was chosen for comparison of glucose transport rate by untreated and treated cells.

Cells were seeded into 25-cm^2 flasks, treated, and incubated in the same manner as for ^{18}F-FDG incorporation. Before the addition of ^{3}H-OMG, medium was replaced with 1 mL of fresh medium for 5 min, and then all medium was removed. A total of 1 mL of ^{3}H-OMG in medium (18.5 kBq/mL) at 37°C was added to inverted flasks, which were rapidly reinverted to allow contact with the cells and begin incubation with ^{3}H-OMG. Once the exposure time was reached, the cells were rapidly washed in ice-cold (0°C) PBS containing phloretin, a glucose transport inhibitor, to inhibit efflux of ^{3}H-OMG. Control and treatment flasks were interleaved throughout the uptake determination to ensure that all were handled in the same manner.

Cells were then trypsinized, and after the addition of medium (final volume, 1 mL) they were mixed; 0.4 mL was added to 20-mL scintillation vials (Packard Biosciences BV) containing 5 mL of scintillation liquid (Liquid Scintillation Cocktail; Meridian) and placed in the dark for 24 h. The ^{3}H-OMG activity present in each sample was then measured for 10 min on a scintillation counter (1900CA Tri Carb Liquid Scintillation Analyser; Packard). Results were expressed relative to total protein. Protein content was determined on another 0.4-mL cell sample, as described for ^{18}F-FDG incorporation.

HK Activity

Following the method of Miccoli et al. (*18*), confluent treated and control cells in 25-cm^2 flasks were washed with PBS and trypsinized. After the addition of medium and transfer to a

microcentrifuge tube, cells were centrifuged at 800*g* for 5 min. The pellet was resuspended in 0.2 mL of homogenization buffer (10 mM of Tris-HCl, pH 7.7; 0.25 mM of sucrose; 0.5 mM of dithiothreitol; 1 mM of aminohexanoic acid; and 1 mM of phenylmethanesulfonyl fluoride) and then homogenized with 10 strokes in a 1-mL Dounce homogenizer (Fisher UK) at 4°C. The homogenized cells were then transferred to a microcentrifuge tube (Eppendorf) and centrifuged at 1,000*g* for 10 min to remove cell debris. The supernatant was then transferred to a new microcentrifuge tube, the pellet was washed with 0.2 mL of homogenization buffer, and the supernatant was pooled. The protein content of the homogenate was determined for a 20-μL sample.

Enzyme activity was determined by the addition of 100 μL of homogenate to 0.9 mL of assay medium consisting of 100 mM of Tris-HCl (pH 8.0), 10 mM of glucose, 0.4 mM of nicotinamide adenine dinucleotide phosphate$^+$ ($NADP^+$), 10 mM of magnesium chloride, 5 mM of ATP, and 0.15 units of glucose-6-phosphate dehydrogenase in a cuvette at 37°C. The reaction was followed by monitoring the change in absorbance at 340 nm due to the formation of NADPH. Enzyme activity was converted to mU/min/mg protein by using the extinction coefficient for NADPH (6.3×10^3 mol^{-1} cm^{-1}).

Annexin Binding

Flasks of cells were set up and treated as for ^{18}F-FDG incorporation. After 72 h, they were trypsinized and collected into microcentrifuge tubes, centrifuged at 400*g* for 5 min at room temperature, and washed with PBS. They were then stained for detection of apoptosis using an Annexin V–Fluorescein Isothiocyanate Apoptosis Detection Kit (Sigma-Aldrich), according to the manufacturer's directions. Cells that were annexin V–positive (annexin V+)/propidium iodide (PI)–negative (PI−) were recognized as early apoptotic; cells that were PI-positive (PI+) were recognized as necrotic.

ATP Content

The ATP assay was performed on treated and control cells using an ATP Bioluminescence Assay Kit (Sigma), according to the manufacturer's instructions. The amount of light emitted was measured with a plate reader (Wallac).

Statistics

Results were expressed as mean ± SD. Significant differences between means were determined using the Student *t* test.

RESULTS

The IC_{50} for SW620 cells incubated for 72 h with 5-FU, oxaliplatin, and irinotecan was 4, 0.1, and 1 μM, respectively; the IC_{50} for HCT-8 cells incubated for 72 h with cetuximab, irinotecan alone, and cetuximab (20 mg/mL) was 50 μg/mL, 2 μM, and 0.2 μM, respectively. Treatment of either cell type with irinotecan for 72 h decreased their colony-forming ability to zero. Treatment of SW620 cells with IC_{50} doses of 5-FU or oxaliplatin decreased the proportion of colony-forming cells to 37% and 43%, respectively.

Percentages of apoptosis (annexin V+ and PI−) and necrosis (annexin V+ and PI+) after 24, 48, and 72 h are shown in Table 1. Apoptosis was not appreciably evident in the HCT-8 cells. In SW620 cells, irinotecan treatment induced apoptosis, which increased with treatment time consistent with the presence of a sub-G1 peak in the cell cycle distribution. Annexin V binding to SW620 cells is also evident in cells treated for 72 h with 5-FU.

^{18}F-FDG Incorporation

^{18}F-FDG incorporation by control and treated SW620 and HCT-8 cells is shown in Figure 1. Compared with untreated cells, ^{18}F-FDG incorporation was significantly decreased by SW620 cells treated for 72 h with 5-FU ($t_{14} = 3.67$, $P < 0.01$), oxaliplatin ($t_{14} = 3.01$, $P < 0.01$), and irinotecan ($t_{14} = 5.56$, $P < 0.001$) and by HCT-8 cells treated with irinotecan ($t_{11} = 5.91$, $P < 0.005$), cetuximab ($t_{11} = 4.54$, $P < 0.005$), and cetuximab plus irinotecan ($t_{11} = 8.0$, $P < 0.005$). However, ^{18}F-FDG incorporation by SW620 cells was increased by shorter exposure times to 5-FU for 24 h ($t_{14} = 5.37$, $P < 0.001$) and 48 h ($t_{14} = 2.85$, $P < 0.01$), to oxaliplatin for 48 h ($t_{14} = 2.96$, $P < 0.01$), and to irinotecan for 24 h ($t_{14} = 2.87$, $P < 0.01$). Compared with controls, ^{18}F-FDG incorporation was decreased in HCT-8 cells treated with cetuximab alone for 24 h ($t_7 = 5.9$, $P < 0.01$) or 48 h ($t_{10} = 6$, $P < 0.005$) or in com-

TABLE 1
Percentage of Apoptosis and Necrosis in Control and Treated SW620 and HCT-8 Cells

Treatment	Time (h)	Viable cells (annexin V− and PI–)	Apoptotic (annexin V+ and PI−)	Necrotic (annexin V+ and PI+)
HCT-8 cells				
Control	24	74	2	23
	48	68	2	30
	72	63	2	34
Cetuximab	24	78	2	20
	48	72	2	26
	72	74	2	24
Irinotecan	24	62	3	35
	48	65	4	31
	72	72	4	25
Irinotecan and cetuximab	24	65	2	31
	48	59	1	40
	72	77	2	21
SW620 cells				
Control	24	70	4	25
	48	88	2	10
	72	86	2	12
5-fluorouracil	8	90	2	7
	24	67	5	27
	34	65	12	23
	48	54	17	29
	72	44	14	42
Oxaliplatin	24	72	4	24
	48	76	5	20
	72	74	7	19
Irinotecan	24	71	7	21
	34	48	3	49
	48	51	10	38
	72	37	19	45

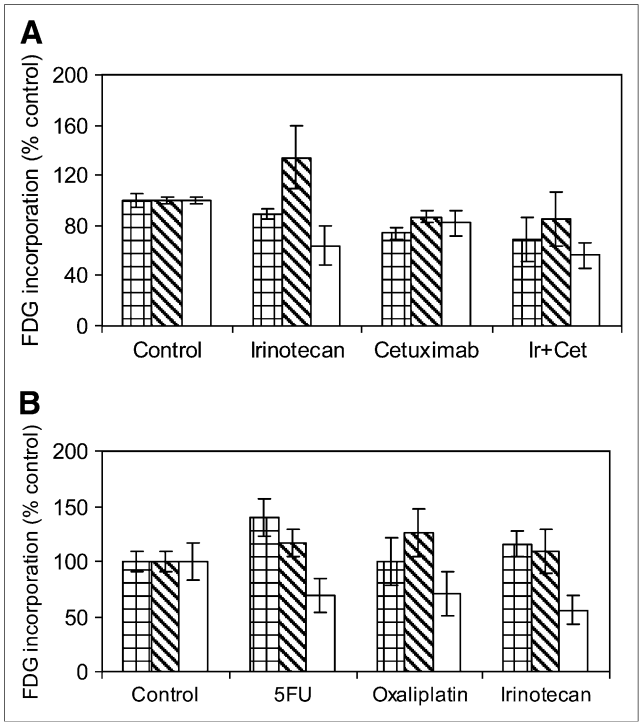

FIGURE 1. ^{18}F-FDG incorporation into HCT-8 cells treated for 24 (n = 3), 48 (n = 6), and 72 h (n = 7) vs. incorporation by untreated controls (n = 6) (A) and into SW620 cells treated for 24 (n = 8), 48 (n = 8), and 72 h (n = 8) vs. incorporation by untreated control cells (n = 8) (B). Checkered bars represent 24-, hatched bars represent 48-, and white bars represent 72-h treatments. Ir+Cet = irinotecan plus cetuximab.

bination with irinotecan for 24 h (t_7 = 2.95, $P < 0.05$) or 48 h (t_{10} = 0.95, not significant); ^{18}F-FDG incorporation was increased by cells treated with irinotecan for 48 h (t_{10} = 3.43, $P < 0.01$).

Glucose Transport

Glucose transport by control and treated SW620 and HCT-8 cells is shown in Figure 2. Glucose transport was significantly changed only in SW620 cells treated for 72 h with oxaliplatin (t_6 = 4.22, $P < 0.02$), compared with untreated SW620 cells. Glucose transport was decreased in HCT-8 cells treated with irinotecan for 24 h (t_8 = 2.62, $P < 0.05$); with cetuximab for 24 (t_8 = 3.04, $P < 0.05$), 48 (t_7 = 2.76 $P < 0.05$), and 72 h (t_{10} = 3.5 $P < 0.01$); and with irinotecan plus cetuximab for 24 (t_8 = 2.47, $P < 0.05$) and 72 h (t_{10} = 2.55, $P < 0.05$).

HK Activity

HK activity in control and treated HCT-8 and SW620 cells is shown in Figure 3. HK activity in treated HCT-8 cells, compared with untreated HCT-8 cells, was increased after 24 (t_5 = 4.29, $P < 0.02$) and 48 h (t_6 = 5.28, $P < 0.01$) with irinotecan, 24 (t_5 = 3.54, $P < 0.05$) and 72 h (t_6 = 3.84, $P < 0.05$) with cetuximab, and 24 (t_5 = 3.27, $P < 0.05$) and 48 h (t_6 = 2.95, $P < 0.05$) with irinotecan plus cetuximab. HK activity was increased by treatment of SW620 cells with 5-FU for 24 h (t_{10} = 3.33, $P < 0.02$) and decreased by treatment with 5-FU (t_{15} = 3.15, $P < 0.01$), oxaliplatin (t_{15} = 2.8, $P < 0.02$), and irinotecan (t_{15} = 4.51, $P < 0.001$) after 72 h.

ATP Content

ATP content, shown in Figure 4, was significantly increased in HCT-8 cells treated for 72 h (t_4 = 7.1, $P < 0.002$) and in SW620 cells treated for 48 (t_7 = 3.58, $P < 0.05$) and 72 h (t_{11} = 2.32, $P < 0.05$) with irinotecan. Treatment of SW620 cells for 72 h with oxaliplatin resulted in decreased ATP content (t_{11} = 3.79, $P < 0.01$).

^{18}F-FDG Incorporation and HK Activity Induced by 8-Hour Drug Treatment

Some drugs are administered to patients over periods of only a few hours. To simulate these clinical situations more closely, SW620 cells were treated for 8 h with IC_{50} drug doses, and then ^{18}F-FDG incorporation was determined either immediately or after 64 h in the presence of drug-free medium. Treatment for 8 h with 5-FU, oxaliplatin, or irinotecan followed by incubation for 64 h in drug-free medium was associated with a decrease in cell number to

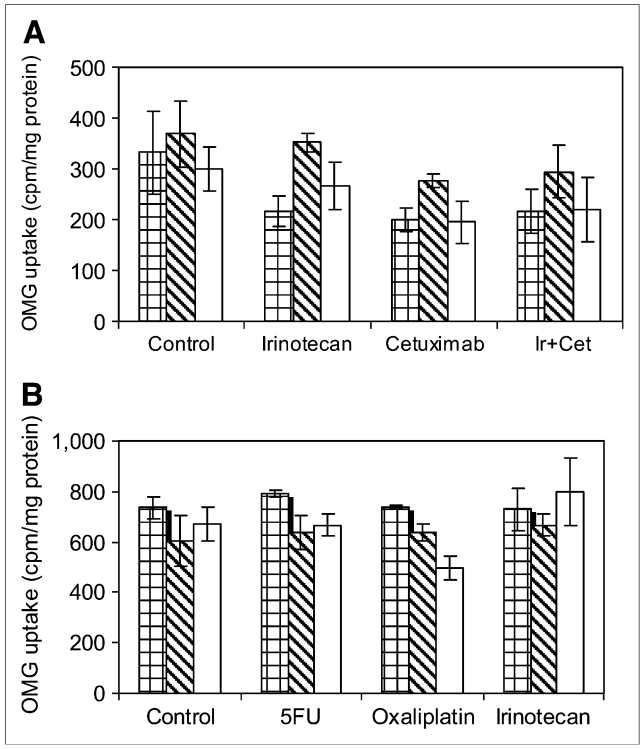

FIGURE 2. Glucose transport (^{3}H-OMG uptake during 3-s [HCT-8 cells] or 5-s [SW620 cells] incubation with ^{3}H-OMG) in HCT-8 cells treated for 24 (n = 4), 48 (n = 3), and 72 h (n = 6) vs. incorporation by untreated controls (n = 6) (A) and in SW620 cells treated for 24 (n = 3), 48 (n = 5), and 72 h (n = 4) vs. incorporation by untreated control cells (n = 4) (B). Checkered bars represent 24-, hatched bars represent 48-, and white bars represent 72-h treatments. cpm = counts per minute; Ir+Cet = irinotecan plus cetuximab.

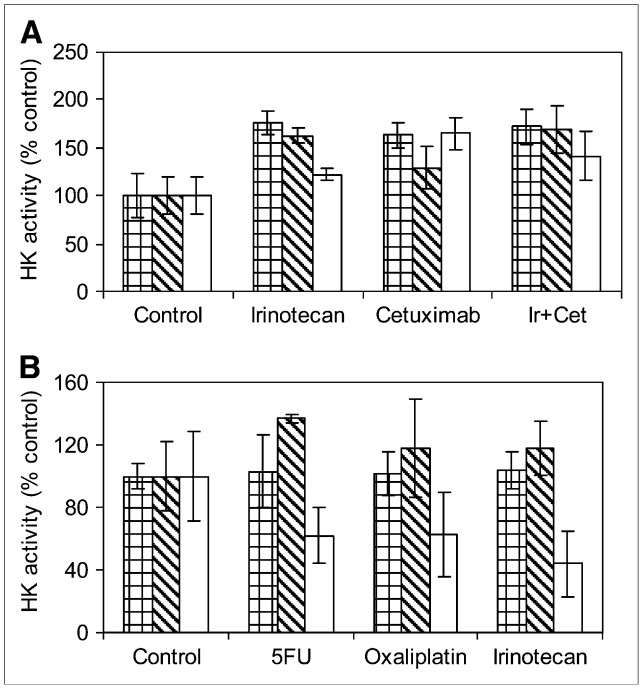

FIGURE 3. HK activity in HCT-8 cells treated for 24 ($n = 3$), 48 ($n = 4$), and 72 h ($n = 4$) vs. incorporation by untreated controls ($n = 4$) (A) and in SW620 cells treated for 24 ($n = 4$), 48 ($n = 4$), and 72 h ($n = 9$) vs. incorporation by untreated control cells ($n = 8$) (B). Checkered bars represent 24-, hatched bars represent 48-, and white bars represent 72-h treatments. Ir+Cet = irinotecan plus cetuximab.

85%, 75%, and 60%, respectively, whereas clonogenic potential was 78%, 40%, and 39%, respectively, compared with untreated cells. The results in Figure 5 show that ^{18}F-FDG incorporation by the cells at the end of the 8-h treatment period was similar to that of the controls but decreased by treatment with 5-FU ($t_4 = 3.8$, $P < 0.01$), oxaliplatin ($t_4 = 2.8$, $P < 0.05$), or irinotecan ($t_4 = 3.55$, $P < 0.01$) when ^{18}F-FDG incorporation was determined 64 h after the end of the 8-h drug treatment. HK activity was decreased to 89% ($t_4 = 4$, $P < 0.05$), 80% ($t_4 = 7.4$, $P < 0.01$), and 83% ($t_4 = 2.8$, $P < 0.05$), respectively, by 5-FU–, oxaliplatin-, or irinotecan-treated SW620 cells, compared with untreated cells, when measured 64 h after completion of the treatment. ATP was unchanged in cells 64 h after incubation in drug-free medium subsequent to treatment for 8 h with 5-FU and irinotecan but significantly ($t_4 = 4.0$, $P < 0.05$) decreased in oxaliplatin-treated cells (28,000 ng of protein per milligram), compared with untreated cells (51,000 ng of protein per milligram).

^{18}F-FDG Incorporation After Glucose Transport Inhibition

Glucose transport and ^{18}F-FDG incorporation by HCT-8 and SW620 cells were determined in the presence of the glucose transport inhibitor cytochalasin B (50 μM). ^{3}H-OMG uptake over 3 s for HCT-8 cells was 275 ± 38 and over 5 s for SW620 cells it was 560 ± 30; in the presence of cytochalasin B, however, uptake decreased to 137 ± 12 ($t_4 = 6.05$, $P < 0.01$) and 219 ± 27 ($t_4 = 7.4$, $P < 0.001$), respectively, demonstrating its inhibitory effect on glucose transport. ^{18}F-FDG incorporation by HCT-8 over a standard 20-min period was 29,087 ± 1,179, but in the presence of cytochalasin B this was greatly decreased to 12,385 ± 2,523 ($t_4 = 10.3$, $P < 0.001$). ^{18}F-FDG incorporation by SW620 cells was actually increased by treatment with cytochalasin B from 32,126 ± 6,071 to 43,866 ± 2,145, although this was not significant ($t_4 = 3.15$, $P < 0.05$).

DISCUSSION

To investigate ^{18}F-FDG incorporation by colorectal tumor cells during response, IC_{50} doses were determined for each drug over a 72-h treatment period using MTT. The IC_{50} doses after a 72-h exposure of SW620 to 5-FU (4 μM), oxaliplatin (0.1 μM), and irinotecan (1 μM) and HCT-8 cells to irinotecan (2 μM) are comparable with those of previous studies (*19*). Thus, Boyer et al. (*19*) reported IC_{50} doses of 4.3, 0.3, and 3.2 μM for HCT-116 cells treated with 5-FU, oxaliplatin, and irinotecan, respectively,. Colony-forming ability was decreased to zero by treatment for 72 h with

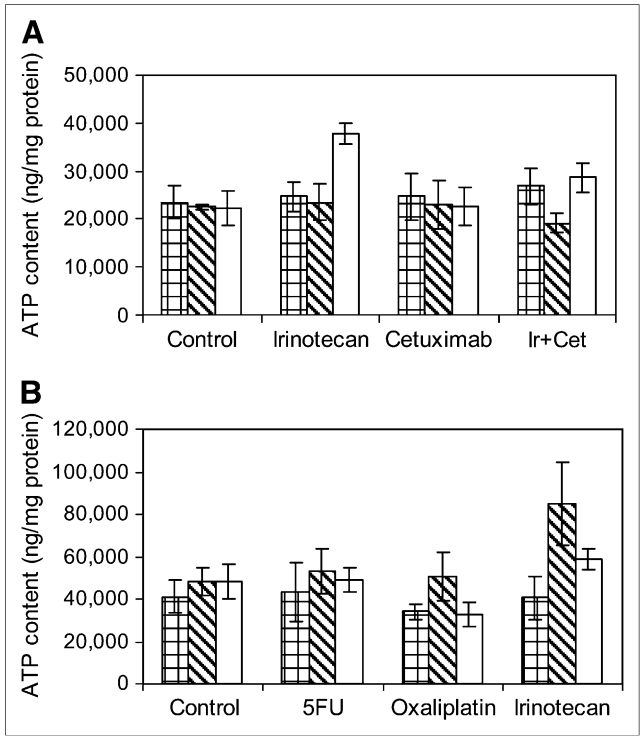

FIGURE 4. ATP content in HCT-8 cells treated for 24 ($n = 3$), 48 ($n = 3$), and 72 h ($n = 3$) vs. incorporation by untreated controls ($n = 3$) (A) and in SW620 cells treated for 24 ($n = 3$), 48 ($n = 3$), and 72 h ($n = 6$) vs. incorporation by untreated control cells ($n = 6$) (B). Checkered bars represent 24-, hatched bars represent 48-, and white bars represent 72-h treatments. Ir+Cet = irinotecan plus cetuximab.

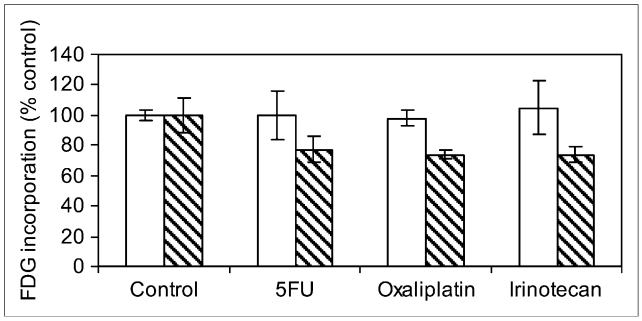

FIGURE 5. ^{18}F-FDG incorporation by SW620 cells treated for 8 h and determined immediately (white bars) or after incubation in drug-free medium for 64 h (hatched bars).

irinotecan, suggesting that the MTT assay underestimated cell death. It also slightly underestimated death of SW620 cells by treatment with 5-FU and with oxaliplatin. Treatment of cells for 8 h followed by incubation in drug-free medium for 64 h also decreased cell number (though unlike with continuous exposure, this was not to 50%) and decreased colony formation, especially after treatment with irinotecan and oxaliplatin. Irinotecan (*20*) is not cell cycle–dependent, whereas oxaliplatin induces effects in both G1 and G2 (*21*). However, 5-FU is an S-phase–specific antimetabolite and would affect a much lower proportion of cells during an 8-h exposure (*22*).

Balin-Gauthier et al. (*15*), using a panel of colorectal carcinoma tumor cell lines consisting of SW620, HT-29, HCT-8, and HCT-116 in ascending order of EGFR expression, found that only the HCT-8 cells exhibited growth inhibition with cetuximab when applied as a monotherapy in vitro using doses of up to 100 μg/mL. They reported that even in this cell line, only a 10% reduction in cell number could be achieved. In contrast, we found that treatment of HCT-8 cells with 50 μg/mL of cetuximab for 72 h inhibited growth by 50%. Coadministration of 20 μg/mL with irinotecan decreased the IC_{50} dose of irinotecan from 1 to 0.2 μM. Our findings of the effect of cetuximab on tumor cell growth are in keeping with the results of several studies that have determined the effect of cetuximab on the growth of cell lines developed from a variety of tumor types in which significant growth-inhibitory effects have been observed. Thus Half et al. (*23*) found that treatment of HCA-7 cells with 7 μg/mL of cetuximab for 48 h reduced viable cell number by 25%, and Xu et al. (*24*) found that treatment of HCT-116 cells and MBA 468 cells with doses of cetuximab up to 20ug/mL for 48 h resulted in decreased cell number by about 30% and 45%, respectively. Raben et al. (*25*) treated a panel of non–small cell lung cancer cell lines with cetuximab (100 nmol/L) over a 6-d period. The cetuximab treatment inhibited the growth of the H292 and H332 cell lines, which had the greatest EGFR expression, by about 75% and the Calu-3 line, which had intermediate EGFR expression, by about 50%. The growth-inhibitory effects of cetuximab on the A549 and H358 lines were more moderate.

Serial clinical PET studies performed on patients with colorectal tumors have demonstrated that decreased ^{18}F-FDG incorporation after treatment, compared with before treatment, corresponds to response (*7–10*). At the tumor cell level, the present study has shown that ^{18}F-FDG incorporation per cell, compared with untreated cells, was decreased by all 72-h treatments in both colorectal tumor cell lines that we examined. Decreased incorporation of ^{18}F-FDG induced by cetuximab in monotherapy and in combination with irinotecan suggests that ^{18}F-FDG PET may be a useful modality in detecting response to treatments that target the EGFR. Some drugs are administered over hours and have blood clearance rates of only a few hours, so ^{18}F-FDG incorporation was also determined in SW620 cells treated for 8 h with each drug either with or without a 64-h recovery period in drug-free medium. In results similar to those for the 72-h continuous exposure experiments, each drug treatment decreased ^{18}F-FDG incorporation when determined 64 h after the 8-h treatment, though not when measured immediately after the treatment period.

In a previous study (*26*), using a 20-fold higher dose of oxaliplatin (2 μM) for 48 h, 2-deoxy-D-[1-^{3}H]glucose incorporation was found to be decreased but only by about 10%. In the present study, treatment of cells with oxaliplatin for 48 h induced insignificant changes in ^{18}F-FDG incorporation at 48 h, probably reflecting the much lower dose used. Increased ^{18}F-FDG incorporation at earlier time points with some treatments was found in cell populations with apoptotic fractions. Thus, irinotecan treatment for 48 h increased ^{18}F-FDG incorporation by SW620 and HCT-8 cells, respectively, and 5-FU treatment for 24 and 48 h increased ^{18}F-FDG incorporation by SW620 cells. Adherent 5-FU– and irinotecan-treated SW620 cells exhibited apoptotic fractions. Treatment of xenografts with ionizing radiation causing apoptosis was accompanied by increased ^{18}F-FDG (*27*). Pelicano et al. (*28*) have recently shown that defects in mitochondrial ATP synthesis result in increased ATP synthesis by glycolysis. Because ^{18}F-FDG incorporation is a measure of glucose use, increased glycolysis would result in increased ^{18}F-FDG incorporation.

Several studies using ^{31}P-nuclear magnetic resonance spectroscopy have shown that nucleotide triphosphate (NTP) (mainly ATP) levels can increase in cells responding to treatment (*29–31*). Thus, NTP levels in the colonic tumor cell line HT-29 cells treated with nitrogen mustard (*29*) and in spheroids of MCF-7 and T47D breast tumor cells treated with doxorubicin during a 24-h period (*30*) increased, but treatment of SW620 cells with doxorubicin did not cause an increase in ATP content (*31*). However, in our study irinotecan treatment was associated with increased ATP levels in SW620 cells after a 48-h and also in HCT-8 cells after a 72-h treatment with irinotecan. This adds further support to the suggestion that increased ATP found after treatment is cell line– or treatment-dependent (*31*). Lutz also suggested that increased ATP levels may reflect apoptosis effects. Irinotecan treatment of SW620 and HCT-8

cells was found to be associated with apoptosis in the present study.

Ferrari et al. (*32*) showed that depletion of ATP content by Jurkat T cells resulted in necrotic cell death rather than apoptosis when subsequently treated with staurosporine or chemotherapeutic drugs. In the present study, decreased ATP was observed only in SW620 cells treated with oxaliplatin, which did not induce apoptosis, in contrast to treatment with irinotecan or 5-FU. Apart from the decrease in ATP content by oxaliplatin-treated SW620 cells, ATP content does not appear to predict cell death. ^{18}F-FDG incorporation after either 72 h of continuous drug exposure or 8 h followed by 64 h in drug-free medium resulted in similar declines in ^{18}F-FDG incorporation; however, the clonogenic capacity was greatly decreased after treatment with irinotecan, compared with 5-FU. ^{18}F-FDG incorporation was decreased in both HCT-8 and SW620 cells treated with irinotecan, but in agreement with the findings of others (*33,34*) apoptosis was only observed in SW620 cells. This suggests that decreased ^{18}F-FDG does not necessarily reflect cellular metabolic changes associated with apoptosis.

Cells that exclude PI but bind annexin V are considered to be in early apoptosis, whereas cells that take in PI and bind annexin V are counted as necrotic or late apoptotic. In the present study, treatment of SW620 cells with both 5-FU and irinotecan increased the number of annexin V+ and PI– cells between 24 and 72 h. However, the proportion of cells that were both annexin+ and PI+ reached higher levels at earlier times, suggesting that necrotic cell death may also be occurring. Alternatively, we may be seeing only necrotic cell death despite observing cells that exclude PI but stain with annexin V because a study of phosphatidylserine exposure during early necrosis has shown that annexin V staining can be observed in the absence of loss of membrane integrity (*35*).

Clonogenic survival is considered the gold standard in determining cytotoxicity because it measures the outcome of all forms of cell death (*36*). However, clonogenicity testing cannot be performed in patients. Recent findings suggest that labeled annexin V imaged using PET or SPECT may detect cell death caused by necrosis and by apoptosis, so it may be an ideal agent for determining total cell death in vivo (*37*). In the present study, ^{18}F-FDG incorporation was shown to be decreased in cells that stained with annexin V and in cells that did not (the oxaliplatin-treated SW620 cells and HCT-8 cells).

In SW620 cells, drug-induced changes in ^{18}F-FDG incorporation more closely paralleled HK activity than did glucose transport, but in HCT-8 cells ^{18}F-FDG incorporation corresponded more closely to glucose transport. The rate-limiting effect on glucose transport in HCT-8 cells was confirmed by measuring ^{18}F-FDG incorporation in the presence of the facilitative glucose transport inhibitor cytochalasin B, which decreased glucose transport in both HCT-8 and SW620 cells but decreased ^{18}F-FDG incorporation only in HCT-8 cells.

In contrast to the 72-h treatments of SW620 cells in which decreased ^{18}F-FDG incorporation parallels decreased HK activity, the increased level of ^{18}F-FDG incorporation induced by the 24-h 5-FU treatment was not accompanied by a corresponding change in HK activity, suggesting that other mechanisms contribute to the early effects of 5-FU on ^{18}F-FDG incorporation by SW620 cells.

The rate-determining step for ^{18}F-FDG incorporation has been shown to differ with tumor type in several studies (*7,13,38–40*). In an ^{18}F-FDG PET study of 21 patients with pancreatic cancer, Higashi et al. (*39*) reported a strong correlation between standardized uptake value at 1 h after injection and glucose transporter 1 (Glut-1) but not HKII protein levels. However, Marom et al. (*40*) showed that in a study of 73 patients with non–small cell lung carcinoma, neither Glut-1 nor Glut-3 protein levels correlated with ^{18}F-FDG incorporation. In a dynamic ^{18}F-FDG PET study of patients with lung and breast tumors, Torizuka et al. (*13*) found that the phosphorylation constant k3 was lower in breast tumors than in lung tumors and correlated with ^{18}F-FDG incorporation in the breast cancer patient group. This finding suggested that it was rate-determining in breast tumors. However, in a more recent and larger study involving 55 patients with breast cancer, Bos et al. (*12*) showed a strong correlation between ^{18}F-FDG and Glut-1 expression.

Our results reveal that even within a single type of cancer (colorectal), the rate-determining step can differ and could account for why clinical studies do not reveal consistent correlates with ^{18}F-FDG incorporation.

In a study by Akhurst et al. (*7*) of ^{18}F-FDG incorporation by 34 metastatic lesions in patients with colorectal tumors, some of whom were undergoing 5-FU–based chemotherapy, ^{18}F-FDG incorporation was lower in lesions in patients who had received chemotherapy, which corresponded to lower HK activity in the treated tumors. In the present study, treatment of SW620 cells with 5-FU for 72 h resulted in decreased HK activity paralleling a decreased ^{18}F-FDG incorporation. In a recent study, Engles et al. (*41*) treated MCF-7 cells for 24 h with 5-FU (200 μM). ^{18}F-FDG incorporation and HKII expression were decreased at the end of the treatment period but Glut-1 expression was enhanced. Differences from our findings may reflect our use of a much lower dose of 5-FU and a different cell line. Differences in HKII and Glut-1 may also reflect our measurement of glucose transport and HK activity at the functional level.

^{18}F-FDG uptake by tumors in vivo may be influenced by blood flow and microenvironments within the tumor, thus confounding the extrapolation of in vitro findings to the clinical situation. However, in vitro studies make a valuable contribution to interpreting clinical findings by clarifying how drugs modulate ^{18}F-FDG incorporation at the tumor cell level and the importance of chemotherapy-induced changes in HK activity and glucose transport in this modulation. The findings of the present study have several

clinical implications. First, the finding that ^{18}F-FDG incorporation is rate-limited by HK in SW620 cells and glucose transport in HCT-8 cells suggests that dynamic studies of tumor ^{18}F-FDG incorporation (*13*) may not necessarily demonstrate either k1 or k3 to be rate-limiting within different tumors of the same cancer type. Second, decreased ^{18}F-FDG incorporation by tumor cells after each 72-h treatment, compared with untreated cells, corresponds to the general finding of lower uptake by tumors exhibiting good response to therapy observed in patient studies. This finding suggests that clinical ^{18}F-FDG tumor studies are observing metabolic changes in tumor cells during response. Last, the fact that ^{18}F-FDG incorporation is not necessarily decreased at time points earlier than 72 h emphasizes the importance of the timing of the posttherapy ^{18}F-FDG scans in clinical studies.

CONCLUSION

We have treated 2 colorectal tumor cell lines with IC_{50} doses of conventional and novel chemotherapy agents used in the treatment of colorectal cancer. In 1 cell line, modulation of glucose transport corresponded to therapy-induced changes in ^{18}F-FDG incorporation; in the other, effects on HK activity more closely corresponded. Changes in ^{18}F-FDG incorporation corresponded qualitatively though not quantitatively with decline in cell number and clonogenic capacity.

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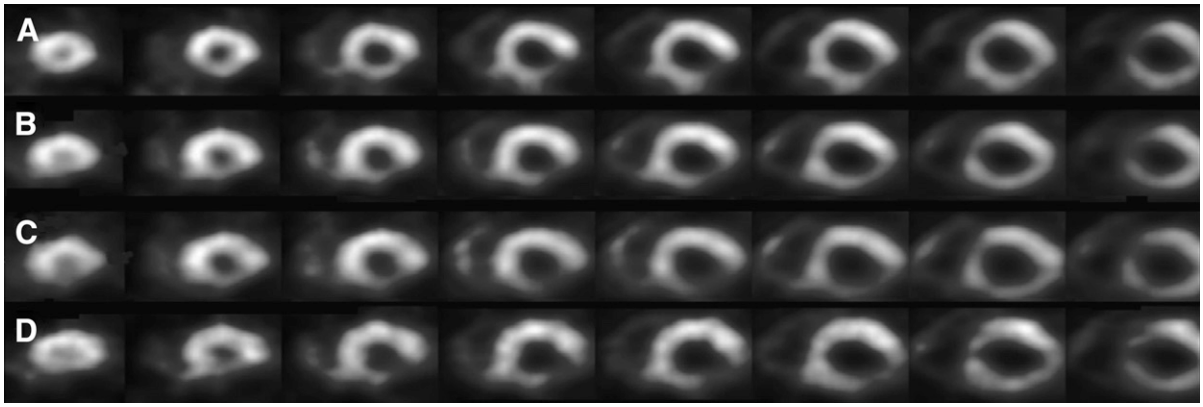
Erratum

In the article "Comparison of Simultaneous Dual-Isotope Multipinhole SPECT with Rotational SPECT in a Group of Patients with Coronary Artery Disease," by Steele et al. (*J Nucl Med.* 2008;49:1080–1089), the vertical and horizontal resolutions of Figure 7 were unequal. The corrected figure appears below. We regret the error.



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Colorectal Tumor Cells Treated with 5-FU, Oxaliplatin, Irinotecan, and Cetuximab Exhibit Changes in ^{18}F-FDG Incorporation Corresponding to Hexokinase Activity and Glucose Transport

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