Changes in 2-fluoro-2-deoxy-D-glucose incorporation, hexokinase activity and lactate production by breast cancer cells responding to treatment with the anti-HER-2 antibody trastuzumab

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Abstract

Introduction: Changes in 2-[^{18}F]-fluoro-2-deoxy-D-glucose (FDG) incorporation by tumors, detected using positron emission tomography, during response to chemotherapy are utilized clinically in patient management. Here, the effect of treatment with growth-inhibitory doses of the anti-human epidermal growth factor receptor-2 antibody trastuzumab (Herceptin) on the incorporation of FDG by breast tumor cells was measured along with hexokinase (HK) and glucose transport to determine the potential of FDG-positron emission tomography in predicting response to these biological anti-cancer therapies and their modulatory effects on the steps involved in FDG incorporation.
Methods: The sensitivity to trastuzumab of three breast tumor cell lines, SKBr3, MDA-MB-453 and MDA-MB-468, expressing human epidermal growth factor receptor-2 at high, medium and low levels, respectively, was determined using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay over a 6-day period, and a clonogenic assay was carried out after 7- and 10-day exposures. FDG incorporation by cells treated with growth-inhibitory doses of trastuzumab was carried out after 4 h and 2, 4 and 6 days of treatment. Glucose transport (rate of uptake of the non-metabolizable analogue [^{3}H]O-methyl-D-glucose), HK activity and lactate production were measured on cells treated with inhibitory doses of trastuzumab for 6 days.
Results: The IC$_{50}$ doses for SKBr3 and MDA-MB-453 and the IC$_{20}$ dose for MDA-MB-468 after 6 days of treatment with trastuzumab were 0.25, 1 and 170 μg/ml, respectively. FDG incorporation by SKBr3 and MDA-MB-453 cells was found to be decreased using IC$_{50}$ doses of trastuzumab for 6 days. At the IC$_{50}$ doses, FDG incorporation was also decreased at 4 days and, in the case of MDA-MB-453, even after 4 h of treatment. Decreased FDG incorporation corresponded with decreased HK activity in these cells. Lactate production, previously suggested to be a potential measure of response, was found to be significantly decreased by SKBr3 and MDA-MB-453 cells responding to trastuzumab.
Conclusion: FDG incorporation at the tumor cell level is modulated by treatment with growth-inhibitory doses of trastuzumab due to modulation of HK activity. Changes in lactate production may also be a useful determinant of response to trastuzumab.
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Keywords: Trastuzumab; FDG; PET; Hexokinase; Lactate

1. Introduction

The human epidermal growth factor receptor (HER) tyrosine kinase family consists of four members (HER-1–HER-4) involved in controlling cellular events, including cell proliferation, differentiation and cell death. The amplification of HER-2 (ErbB2/*neu*), observed in several cancer types [1], is associated with resistance to apoptosis, enhanced proliferation, cell migration and invasiveness. About 25% of cases of breast cancer exhibit HER-2 overexpression, which is strongly associated with poor prognosis [2]. These patients derive clinical benefit from treatment with the HER-2-targeting antibody trastuzumab (Herceptin) [3]. However, trastuzumab is very expensive,

and it produces cardiac complications [4] in some patients, especially when administered with anthracyclines. Furthermore, HER-2-negative cells have been shown to exhibit little or no response to the drug [5].

It is recommended [6] that overexpression of HER-2 should be evaluated on every primary breast cancer to predict which patients may benefit from trastuzumab. This is determined using either fluorescent in situ hybridization or immunohistochemistry on biopsy specimens taken from the primary tumor. However, this approach may not provide globally representative information on either the primary tumor or metastasis, which can exhibit an HER-2 status different from the primary tumor [7], or the likelihood of its response to trastuzumab.

An alternative approach to tumor therapy response prediction is the use of positron emission tomography (PET) to monitor changes in tracer incorporation during the course of treatment. 2-[^{18}F]-Fluoro-2-deoxy-D-glucose (FDG) is the most commonly utilized PET tracer with availability at all PET centers. The use of serial FDG-PET scans in cancer patients during the course of chemotherapy has been demonstrated by many studies to be a useful tool in cancer management [8]. Generally, compared with pretreatment, responding tumors show decreased FDG uptake within a few days of starting chemotherapy. Two recent studies of human tumor xenografts [9,10] have produced conflicting findings regarding the effect of response to trastuzumab on FDG incorporation. McLarty et al. [9] showed that treatment of MDA-MB-361 cells, which overexpress HER-2, exhibited a decline in FDG incorporation, whereas MDA-MB-231 cells, which do not overexpress HER-2, showed no change in FDG incorporation. However, in the study by Shah et al. [10], FDG incorporation by trastuzumab-treated MMTV/HER-2 and BT474 cells was found not to accompany response. Neither study carried out in vitro studies to assist in understanding the effect of trastuzumab FDG incorporation at the tumor cell level.

To understand the importance of time and dose of anti-HER-2 antibodies on FDG incorporation at the tumor cell level, we have measured FDG incorporation in the breast tumor cells SKBr3 [which overexpress HER-2 at a high level (900 ng/mg)], MDA-MB-453 [which overexpress HER-2 at a medium level (100 ng/ml)] and MDA-MB-468 (which do not overexpress HER-2) [11] at various time points during treatment with growth-inhibitory doses of trastuzumab. Akt is a downstream effector of HER-2 and is known to influence the activity of hexokinase (HK) [12] and mitochondrial membrane potential. Therefore, we determined HK activity and lactate production during treatment with trastuzumab. Glucose transport was also measured due to its important role in FDG uptake. Growth inhibition was determined using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assays and verified using the clonogenic assay, the gold standard for measuring therapeutic efficacy.

2. Materials and methods

All chemicals were obtained from Sigma-Aldrich (Poole, UK) unless otherwise stated. Cell lines were obtained from American Type Culture Collection (Manassas, VA, USA).

2.1. Cells and treatments

SKBr3, MDA-MB-453 and MDA-MB-468 cells were cultured in DMEM (with Glutamax) (Invitrogen, Paisley, UK) and supplemented with 50 U/ml of penicillin, 50 µg/ml of streptomycin and 10% fetal bovine serum. During drug incubation periods for the FDG uptake, glucose transport, HK and lactate assays, media changes were carried out on Day 4.

2.2. Cell viability

2.2.1. MTT assay

Cells were grown until confluent in 75-cm^2 flasks, and after trypsinization and addition of medium, the cell numbers were corrected to 15,000/ml for the SKBr3 and MDA-MB-453 cells and 7500/ml for the faster-growing MDA-MB-468 cells. One hundred microliters of cell suspension was seeded into 96-well plates, and the plates were incubated at 37°C overnight. Medium (100 µl) supplemented with varying concentrations of trastuzumab to produce final concentrations within the ranges of 0.001–0.25 µg/ml for SKBr3, 0.025–5 µg/ml for MDA-MB-453 and 1–250 µg/ml for MDA-MB-468 was then added to each well (200 µl/well in total), and the plate was returned to the incubator. A background of 200 µl of medium and a control of 200 µl of medium and cells were also set up. After 6 days, 50 µl of MTT was added to each of the wells and incubated for 3–4 h. The media were then aspirated using a syringe, and 200 µl of dimethylsulfoxide was then added to each well. Each plate was then placed in a scanning multi-well spectrophotometer (Dynatech MR5000, Dynatech Laboratories, Chantilly, VA, USA) and agitated for 30 s prior to measuring spectrophotometric absorbance at 570 nm (test filter=570 nm, reference filter=690 nm). The plates were analyzed using Biolinx 2.0 software (Biolinx 2.0, Dynatech Laboratories). The absorbance at each treatment concentration was calculated as a percentage of that for the control cells. The MTT assay was carried out in four separate experiments, the data were pooled and a curve of trastuzumab concentration vs. relative absorbance was used to determine IC values (Fig. 1).

2.2.2. Colony formation

The ability of SKBr3 and MDA-MB-453 cells treated continuously with trastuzumab (IC$_{20}$ and IC$_{50}$) and MDA-MB-468 cells treated with trastuzumab (IC$_{10}$ and IC$_{20}$) to proliferate was determined by seeding cells into 25-cm^2 tissue culture flasks (20,000 per flask) and determining the number of cells that form colonies of at least 25 cells after 7 days and at least 50 cells by 10 days (results are expressed relative to the number in control flasks). Colony counts were
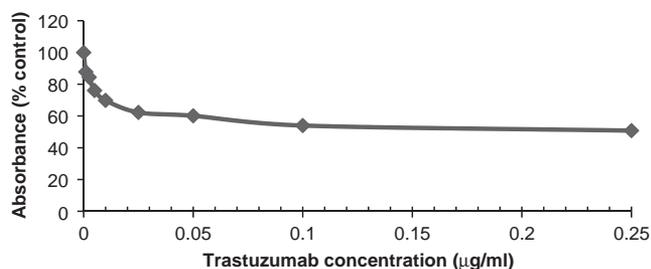
Fig. 1. MTT incorporation (absorbance at 570 nm) relative to untreated cells (%) by SKBr3 cells treated for 6 days with a range of concentrations of trastuzumab.

carried out using a Nikon Eclipse TS100 inverting microscope with a 10× objective lens (individual cells could be clearly seen using this lens) counting cells in 20 fields per flask. The total colonies per flask were then expressed as the percentage of total colonies formed in the control flasks. Controls and all treatments were set up with four replicates.

2.2.3. FDG incorporation

Cells were seeded in 25-cm^2 tissue culture flasks and 3 days later treated with requisite doses of trastuzumab for 4 h, 2 days, 4 days and 6 days. At the time of FDG uptake determination, medium was replaced with 1 ml of fresh medium [glucose concentration=1 mg/ml (5 mM), as this is about the same glucose concentration found in serum from a non-diabetic subject] containing 37 KBq of FDG and incubated for 20 min at 37°C. Cells were then washed five times with 5 ml of ice-cold PBS and detached by addition of trypsin. After addition of medium to neutralize the trypsin, 0.8 ml of cell suspension was transferred into microcentrifuge (Eppendorf) tubes for determination of FDG uptake in a well counter. The cells were then centrifuged and washed with 1 ml of PBS, the pellet was dissolved in 0.1 ml NaOH (1 M) and protein content was determined after neutralizing with 0.1 ml HCl. Protein content was determined using a bicinchoninic acid protein assay kit (Sigma, Poole, UK). The uptake of FDG was normalized by dividing by protein content. Uptake by treated cells was then expressed as the percentage of uptake by control cells.

2.2.4. Glucose transport

Glucose transport rate was determined by incubating cells with [^{3}H]O-methyl-D-glucose ([^{3}H]OMG), and the amount taken up before equilibrium occurred was considered to be a measure of glucose transport, determined.

Flasks of cells were seeded as for FDG uptake. At the time of glucose transport determination, medium was removed and replaced with fresh medium (glucose concentration=1 mg/ml) containing 37 KBq of [^{3}H]OMG and 0.1 mM "cold" OMG for 5 and 10 s. The incubation was ended by rapid addition of 5 ml of ice-cold PBS containing the glucose transport inhibitor phloretin (0.1 mM) followed

by three further rapid washes. Cells were then trypsinized, half of them added to 5 ml of Optima Gold scintillation fluid (Perkin Elmer, UK), and [^{3}H]OMG uptake was determined in a scintillation counter. The remaining cells were prepared for protein assay. The uptake of [^{3}H] during the 5- and 10-s periods was normalized by dividing by total protein content of cells in each flask. Values for the treated cells were then expressed as the percentage of uptake by control cells.

2.2.5. HK activity

Cells were seeded in 75-cm^2 flasks and after treatment with trastuzumab for 6 days harvested by trypsinization. After addition of medium, they were collected into microcentrifuge (Eppendorf) tubes, washed with PBS and centrifuged at 400g for 1 min, and the pellet was resuspended in 0.2 ml of homogenization buffer (10 mM Tris–HCl, pH 7.7, 0.25 mM sucrose, 0.5 mM dithiothreitol, 1 mM aminohexanoic acid and 1 mM PMSF). They were then transferred to a 1-ml glass Dounce homogenizer and homogenized by 10 strokes at 4°C. The homogenized cells were transferred to a microcentrifuge tube and centrifuged for 10 min at 800g to remove cell debris. The supernatant was transferred to a new microcentrifuge tube, the pellet was washed with 0.2 ml of homogenization buffer and the supernatant was pooled. Protein content of the homogenate was determined on a 20-μl sample.

Enzyme activity was determined by addition of 100 μl of homogenate to 0.9 ml of assay medium consisting of 100 mM Tris–HCl, pH 8.0, 10 mM glucose, 0.4 mM NADP$^+$, 10 mM MgCl$_2$, 5 mM ATP and 0.15 U 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. The change in absorbance was normalized by dividing by protein content of the homogenate. HK activity by treated cells was then expressed as the percentage of HK activity of control cells.

2.2.6. Lactate production

Cells were set up and treated for 6 days as for glucose uptake determination. The cells were then washed with PBS (4×) and then incubated for 30 min with DMEM medium (containing 1 mg/ml of glucose). The medium was then removed and stored at −20°C until lactate was assayed. Cells were then trypsinized and prepared for protein assay as for FDG incorporation. Lactate was assayed in the medium using a lactate assay kit (BioVision, CA, USA) following the manufacturer's directions. Lactate concentration in nanomoles produced during the 30-min incubation was normalized by dividing by protein content of the flask. Production of lactate by treated cells was expressed as a percentage of that by control cells.

2.3. Statistics and replicates

Significant differences between means were established using Student's t test. Values are included in the text and are

Table 1
Growth-inhibitory doses (μg/ml) for breast tumor cells treated with trastuzumab for 6 days determined using the MTT assay

	SKBr3	MDA-MB-453	MDA-MB-468
IC_{50}	0.25	1	–
IC_{20}	0.005	0.015	170
IC_{10}	–	–	5

written with the number of degrees of freedom [=Total Number of Replicates in Control and Treatment Groups−2; e.g., if there are four controls and five treated samples, then the *t* value is expressed as (t_7=x, P<y), and where the difference between the means is not significant, this is described by (t_7=x, ns)]. Experiments were generally carried out using four replicates of treated and control flasks such that the degrees of freedom value is 6. Data from several experiments were combined in most cases, and this is reflected in the degrees of freedom.

3. Results

3.1. MTT and clonogenic assays

Inhibitory doses for trastuzumab incubated for 6 days with MDA-MB-468, MDA-MB-453 and SKBr3 breast tumor cells are shown in Table 1. The maximum achievable inhibition of MDA-MB-468 with trastuzumab was 20% using a mean dose (±S.D.) of 170 (±62) μg/ml, whereas the IC_{50} doses for MDA-MB-453 and SKBr3 were much lower at 1 (±1) and 0.25 (±0.11) μg/ml, respectively.

Fig. 2 shows the effects of growth-inhibitory doses of trastuzumab on the clonogenic potential of each cell line after 7 and 10 days of continuous exposure to each drug. The IC_{20} and IC_{50} doses of trastuzumab determined using the MTT assay inhibited the colony-forming ability of SKBr3 by about 20% (t_6=2.77, P<.05) and 50% (t_6=5.12, P<.01) after 10 days of treatment. The IC_{50} dose also reduced the colony

Table 2
Summary of changes in clonogenic potential after 10 days of treatment and in FDG incorporation, HK activity, glucose transport and lactate production after 6 days of treatment with IC_{50} (SKBr3 and MDA-MB-453) and IC_{20} (MDA-MB-468) doses of trastuzumab

Cell line	Parameter				
	Colonies	FDG	HK activity	Glucose transport	Lactate output
SKBr3	↓	↓	↓	nc	↓
MDA-MB-453	↓	↓	↓	↓	↓
MDA-MB-468	Nc	↓	↓	nc	nc

nc indicates no change.

formation by MDA-MB-453 cells (t_6=3.07, P<.05) by about 50% corresponding with the MTT assay. IC_{10} and IC_{20} doses of trastuzumab on MDA-MB-468 cells had almost no effect on colony formation even after 10 days of treatment [IC_{10} (t_6=0.9, ns) and IC_{20} (t_6=1.3, ns)].

Incorporation of FDG per milligram of protein relative to untreated controls after treatment of SKBr3 and MDA-MB-453 cells with IC_{20} and IC_{50} and that after treatment of MDA-MB-468 cells with IC_{10} and IC_{20} doses of trastuzumab for 4 h, 2 days, 4 days and 6 days relative to untreated cells are shown in Fig. 3. Incorporation of FDG by SKBr3 cells was not significantly decreased by treatment with trastuzumab for 4 h with IC_{20} and IC_{50} doses (t_6=0.56, ns; t_6=0.21, ns) and 2 days with IC_{20} dose (t_{28}=0.29, ns) but was increased with IC_{50} dose for 2 days (t_{28}=4.87, P<.001) and decreased after treatment for 4 days (t_{22}=2.4, P<.05) and 6 days (t_{30}=2.18, P<.05) with IC_{50} dose and 4 days (t_{22}=2.03, P<.05) with IC_{20} dose of trastuzumab by the SKBr3 cells. Incorporation of FDG by trastuzumab-treated MDA-MB-453 cells was not significantly changed by IC_{20} dose for 4 h (t_{14}=1.22, ns), 2 days (t_{21}=1.55, ns), 4 days (t_{12}=0.44, ns) and 6 days (t_{12}=0.36, ns) but was significantly decreased by treatment with IC_{50} dose for 4 h (t_{14}=2.83, P<.01), 2 days (t_{21}=2.07, P<.05), 4 days (t_{12}=2.59, P<.05)
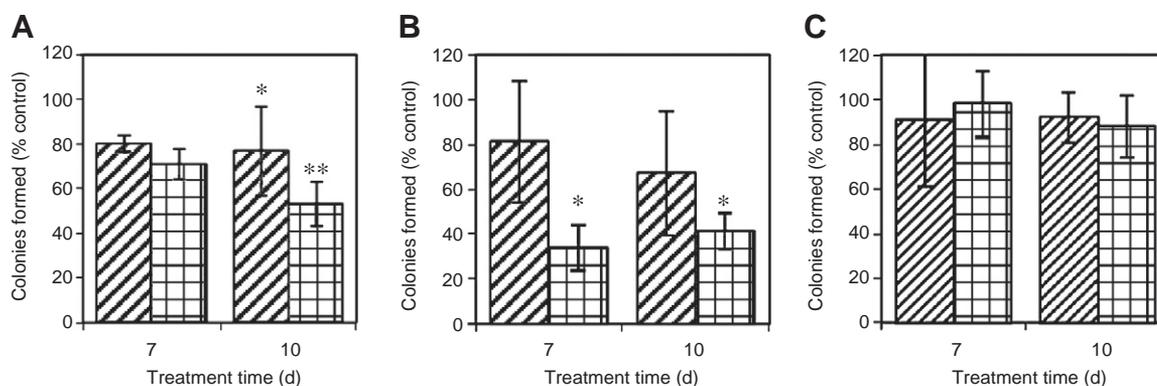

Fig. 2. Formation of colonies of >25 cells (Day 7) or >50 cells (Day 10) by SKBr3 (A), MDA-MB-453 (B) and MDA-MB-468 (C) breast tumor cells treated with low (hatched symbols) (IC_{20} for SKBr3 and MDA-MB-453, IC_{10} for MDA-MB-468) and high (squares) (IC_{50} for SKBr3 and MDA-MB-453, IC_{20} for MDA-MB-468) doses of trastuzumab. Data are expressed as the number of colonies in treated flasks relative to the number of colonies in control flasks (%). The asterisks indicate significant difference from control at *P<.05 and **P<.01.
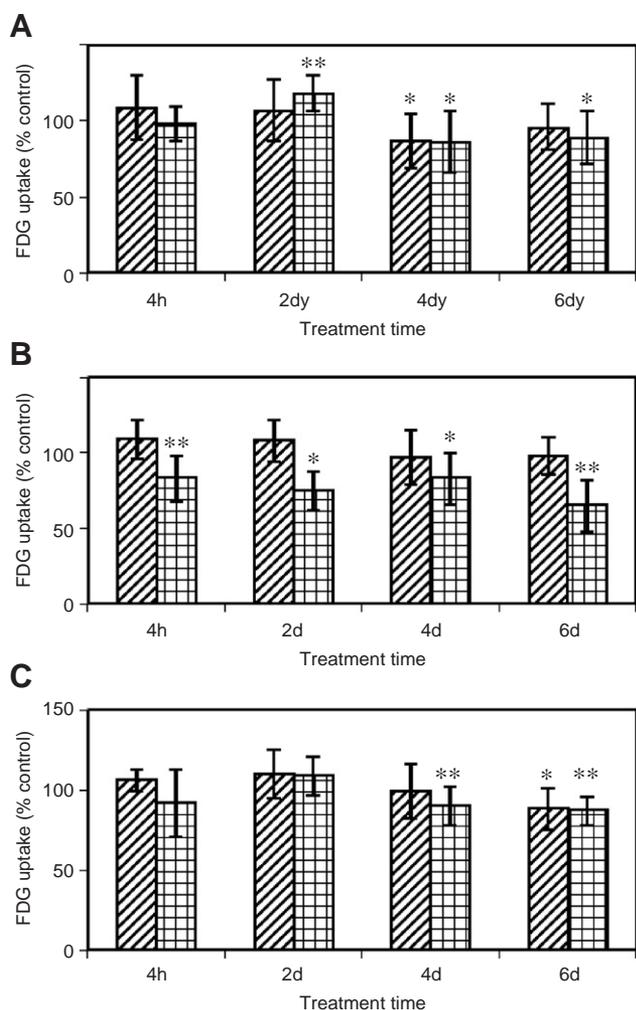
Fig. 3. FDG incorporation per milligram of protein after trastuzumab treatment with (A) SKBr3 [IC_{20} (hatched symbols) and IC_{50} (squares)], (B) MDA-MB-453 [IC_{20} (hatched symbols) and IC_{50} (squares)] and (C) MDA-MB-468 [IC_{10} (hatched symbols) and IC_{20} (squares)]. FDG uptake per flask was normalized to protein content. Normalized uptake by treated cells is expressed relative to normalized uptake by control cells (%). The asterisks indicate significant difference from control at *$P<.05$ and **$P<.01$.
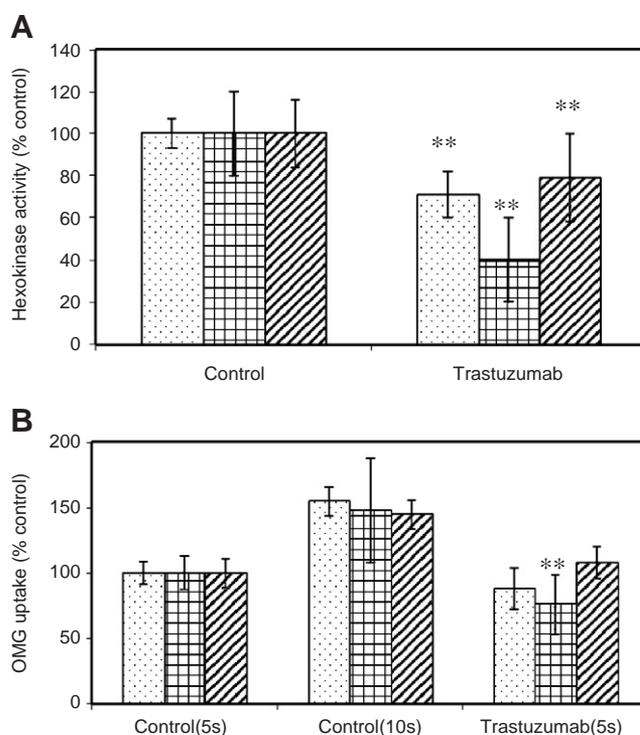

Fig. 4. (A, B) HK activity (A) and glucose transport (B) by SKBr3 (dots), MDA-MB-453 (squares) and MDA-MB-468 (hatched symbols) cells treated with IC_{50}, IC_{50} and IC_{20} doses, respectively, of trastuzumab. Absorbance change per minute (due to NADPH formation) normalized to protein content. Normalized HK activity by treated cells is expressed relative to normalized activity by control cells (%). The double asterisk indicates significant difference from control at $P<.01$.

and 6 days ($t_{16}=6.2$, $P<.01$). FDG incorporation was decreased in MDA-MB-468 cells after 4 days ($t_{18}=2.78$, $P<.01$) and 6 days of treatment with both the IC_{20} dose ($t_{11}=3.01$, $P<.01$) and the IC_{10} dose ($t_{11}=2.12$, $P<.05$) of trastuzumab. The lower doses of trastuzumab did not significantly decrease FDG incorporation at earlier time points of 4 h ($t_{21}=1.7$, ns), 2 days ($t_{14}=1.62$, ns) and 4 days ($t_{18}=0.18$, ns).

HK activity in homogenates prepared from SKBr3, MDA-MB-453 and MDA-MB-468 treated with trastuzumab for 6 days using IC_{50} doses is shown in Fig. 4A as a percentage of HK activity in respective untreated cells. The activity of HK is significantly decreased by SKBr3 ($t_{10}=5.64$, $P<.001$), MDA-MB-453 ($t_{10}=5.78$, $P<.001$) and

MDA-MB-468 ($t_{28}=2.96$, $P<.005$) cells after treatment with trastuzumab compared with control cells. As FDG incorporation was decreased after 4 h of treatment by MDA-MB-453 cells with trastuzumab, HK activity was measured and found to be significantly ($t_8=2.36$, $P<.02$) decreased by about 20% compared with control cells [4 h of trastuzumab treatment, 57 ($\pm$5.8) mU/min/mg of protein; controls, 72 ($\pm$12.7) mU/min/mg of protein].

Fig. 4B shows the uptake of [^{3}H]OMG by untreated cells incubated with [^{3}H]OMG (with 0.1 mM OMG) for 5 s and by cells treated with IC_{50} (SKBr3 and MDA-MB-453) and IC_{20} (MDA-MB-468) doses of trastuzumab for 6 days. ([^{3}H]OMG uptake by control cells incubated for10 s with OMG is also given to show that the uptake of OMG had not reached equilibrium at 5 s.) Glucose transport was found to be significantly decreased by MDA-MB-453 ($t_{19}=2.74$, $P<.01$), but not by MDA-MB-468 ($t_6=0.98$, ns) and SKBr3 ($t_6=1.32$, ns), cells treated for 6 days with trastuzumab compared with untreated cells.

Lactate production was measured in each cell line after treatment for 6 days with IC_{50} doses of trastuzumab. The results in Fig. 5 show that treatment with trastuzumab is accompanied by significant decreases in lactic acid production by SKBr3 ($t_6=2.45$, $P<.05$) and MDA-MB-453
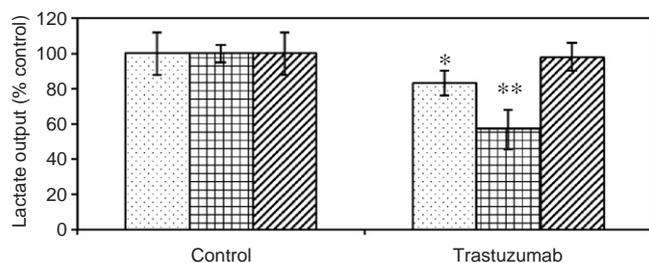
Fig. 5. Lactate production by SKBr3 (dots), MDA-MB-453 (squares) and MDA-MB-468 (hatched symbols) cells treated with IC$_{50}$, IC$_{50}$ and IC$_{20}$ doses, respectively, of trastuzumab. Lactate production by treated cells is expressed relative to control cells (%).The asterisks indicate significant difference from control at *$P<.05$ and **$P<.01$.

(t_{12}=9.42, $P<.001$) cells, but not by MDA-MB-468 cells (t_{12}=0.41, ns) (Table 2).

4. Discussion

The purpose of this study was to determine how FDG incorporation was modulated during response of breast tumor cells to treatment with trastuzumab, which targets HER-2. Three tumor cell lines expressing HER-2 at high, medium and low/negative levels were employed in this study in common with controls utilized in studies of the assessment of suitability of breast tumors for treatment with trastuzumab [13].

In the clinic, trastuzumab is administered to patients on a weekly basis, maintaining a steady-state serum concentration of 50–60 μg/ml [14]. Sensitivity of cells to trastuzumab was determined using the MTT assay after drug exposure over a 6-day period as shorter exposure times did not produce a dose inhibition of 50% even in the most sensitive cell line (SKBr3). The dose of trastuzumab causing 50% growth inhibition was lowest for the SKBr3 cells, which exhibit the greatest overexpression of HER-2 [15]. Growth inhibition of MDA-MB-468 cells, which express normal levels of HER-2, was observed with a 10% inhibition using a dose of 5 μg/ml and 20% using 170 μg/ml. Thus, according to the MTT assay, clinically relevant doses of 50–60 μg/ml would result in some growth inhibition of tumor cells that do not overexpress HER-2.

Although the MTT assay is effective in demonstrating sensitivity of cells to a drug, it is a measure of mitochondrial oxidative potential and utilized as a measure of cell number but does not reveal whether or not those cells are fully functional in terms of long-term proliferative ability. Furthermore, some drug treatments influence mitochondrial oxidative potential. The colony-forming assay, on the other hand, measures a drug's ability to induce clonogenic cell death [16,17]. With the use of this assay, continuous exposure to trastuzumab for 7 and 10 days was found to have produced only a marginal decrease in colony formation after treatment of MDA-MB-468 cells with the apparent

IC$_{20}$ determined using the MTT assay, suggesting that treatment with trastuzumab would not be therapeutically effective as expected for HER-2-negative cells. Continuous exposure of SKBr3 and MDA-MB-453 cells to trastuzumab appreciably decreased their proliferative capacity, concurring with the MTT results and demonstrating the effectiveness of trastuzumab therapy for HER-2-overexpressing tumor cells.

In terms of HER-2 expression, MDA-MB-453 cells are considered to be 2+ [18], which is the minimum expression by breast tumors for which trastuzumab is clinically utilized. Cells that were sensitive to trastuzumab (SKBr3) exhibited decreased FDG incorporation relative to untreated cells when treated with IC$_{50}$ doses of trastuzumab for 4 and 6 days. However, MDA-MB-453 cells, which exhibit lower HER-2 expression and are less sensitive to trastuzumab, exhibited greater decreases in incorporation and at earlier time points.

The response of SKBr3 cells to trastuzumab was accompanied at 2 days by increased FDG incorporation before declining to below control levels. This may reflect an early stimulatory effect of trastuzumab on SKBr3 cells. Metabolic flare is more commonly associated with tamoxifen treatment [19]; however, two studies [20,21] have reported that exposure of tumor cells, including SKBr3 cells, to trastuzumab results in an initial stimulatory phosphorylation of HER-2. Cell death has also been shown to be associated with an early increase in FDG incorporation. Thus, Aide et al. [22] recently showed that FDG incorporation increased 2 days after treatment of testicular tumor xenografts with cisplatin before decreasing at longer time points, which was attributed to events associated with cell death.

Changes in FDG incorporation have been documented in xenografts and in patients responding to the tyrosine kinase inhibitors CI-1033 [23] and lapatinib [24], respectively, which inhibit the epidermal growth factor receptor tyrosine kinases ErbB1 and ErbB2 downstream from HER-1 and HER-2. In a study of eight patients with various solid tumors, one patient who had trastuzumab-resistant Her-2 3+ tumor exhibiting a partial response showed the greatest decrease in FDG incorporation 1 month after the start of treatment [24].

The present study found that decreases at 6 days (and at 4 h of treatment with MDA-MB-453 cells) in FDG incorporation corresponded with decreased HK activity. HK expression is increased by activation of the EGFR/ErbB pathway [25], so inhibition would be expected to decrease incorporation as observed in the present study. These findings also suggest that HK activity in these breast tumor cell lines may be rate-limiting for FDG incorporation, concurring with studies on breast cancer patients [26].

FDG incorporation measures the rate of glucose utilization via glycolysis. The product of glycolysis, pyruvate, can either be converted to acetyl-CoA and enter the tricarboxylic acid cycle or be converted to lactic acid. Lactate levels in

tumors can be monitored using ^{1}H NMR imaging, and studies have shown using this technique that response to therapy can be accompanied by decreased levels of lactic acid [27]. Thus, Lee et al. [27] showed that response to treatment of xenografts derived from WSU-DLCL2 non-Hodgkin's lymphoma cells with a combination of rituximab, cyclophosphamide, hydroxydoxorubicin, vincristine and prednisone exhibited decreased lactate levels. On the other hand, the use of hyperpolarized ^{13}C-labeled pyruvate utilization [28] has shown decreased flux of the hyperpolarized ^{13}C label between pyruvate and lactate, corresponding with decreased FDG uptake by murine lymphoma cells after treatment for 24 h with the topoisomerase II inhibitor, etoposide. In the present study, both SKBr3 and MDA-MB-453 cells exhibited significant decreases in lactate production after treatment with trastuzumab for 6 days, suggesting that following lactate production, pre- and post-treatment may be useful in predicting response to anti-HER-2 antibody treatment.

High doses of trastuzumab were found to induce a moderate growth-inhibitory effect on MDA-MB-468 cells and to significantly decrease FDG incorporation by this cell line, although only by about 10% and at later time points. This could be attributed to an interruption of the pairing effect of HER-2 with other HER receptors by trastuzumab [29,30].

In conclusion, changes in FDG incorporation at the cell level by breast tumor cells correspond with response to IC_{50} doses of the anti-HER-2 antibody trastuzumab. The sensitivity of HK to this drug underlies the reduction in FDG incorporation. Decreased lactate production may be a useful indicator of response to trastuzumab.

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