Metabolic Design of Combination Therapy: Use of Enhanced Fluorodeoxyglucose Uptake Caused by Chemotherapy

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In order to quantify effects of an experimental chemotherapy, MCF7 cells were studied with ^{14}C-fluorodeoxyglucose (FDG) and high-performance liquid chromatography (HPLC). Uptake measurements were performed 1 and 4 hr after the end of a therapy with hexadecylphosphocholine (HPC). A dose- and time-dependent increase of the FDG uptake after therapy was observed, with a maximum at 1 hr after therapy. These data were used to develop a new metabolic design of combination treatment. Several time-dose combinations of HPC and deoxyglucose (DOG) were analyzed for their effects on growth inhibition. The combinations using DOG in the period of pronounced enhancement of FDG uptake (1 hr after HPC treatment) were found to be the most effective with an improvement of up to 520% in growth inhibition. This metabolic design of combination treatment may also be applied in vivo, and PET can be used to optimize the time and dose schedule of the modified treatment protocol.

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The concept of an ordered pattern of enzymic imbalance in neoplastic cells with enhanced activities of several key enzymes has led to the approach of enzyme-pattern targeted chemotherapy (*1–3*). The two steps of this approach are the detection of a specific enzyme pattern of the tumor and then the design of the appropriate therapy. One important application of this principle was the blocking of the pyrimidine de novo synthesis as well as that of the pyrimidine salvage pathway (*3*). Another way to develop a combination therapy may be to measure the time and dose dependence of metabolic changes caused by a therapeutic agent and thereafter to choose a second agent, which is directed against the enzymes involved in these tumor reactions.

Hexadecylphosphocholine (HPC) is suitable for oral application with low bone marrow toxicity (*4,5*). It has a variety of biological effects: some of the proposed mechanisms are interaction and incorporation in cell membranes and partial restructuring of the chromatin, which may contribute to an inhibition of the DNA synthesis (*6*). It has been suggested that the phospholipases C and D may play a role in HPC catabolism and lead to cytotoxic metabolites (*4,7*). The drug is metabolized mainly to choline, phosphocholine and 1,2-diacylphosphatidylcholine. The activity of the phospholipases may be one factor that determines the efficacy of HPC for some tumors (*4,7*). Überall et al. found that HPC inhibits protein kinase C as well as phosphoinositase C and suggested that the growth inhibitory effect of the drug may partly be explained by interference with mitogenic signal transduction (*8*). The drug may act as a competitive inhibitor for phospholipase C-type enzymes. However the exact mechanism of action is not known.

Therapy induced changes in the tumor tissue may be measured in vivo with positron emission tomography (PET) using purpose-designed radiotracers, such as fluorodeoxyglucose (FDG) (*9–16*). This in vitro study was undertaken to study the effects of HPC on the glucose metabolism of breast cancer cells measured by the FDG uptake and to evaluate a metabolic designed therapy, which is based on therapy induced metabolic changes and may enhance the cytostatic effect without increasing the dose of the chemotherapeutic agent.

MATERIALS AND METHODS

Cell Culture

Human breast cancer cells (wild type MCF7 cells) were maintained in culture flasks (Falcon) in RPMI-1640 medium (Gibco BRL, Eggenstein, FRG), supplemented with 10% fetal calf serum, 292 mg glutamine, 100,000 IE penicillin and 100 mg streptomycin/l at 37° C, in an atmosphere of 95% air and 5% CO_2.

FDG Uptake

Cells were trypsinized and 6×10^5 cells were seeded in 6-well plates (Greiner). After adhesion and when the cells reached the logarithmic growth phase, they were incubated for 2 hr or for 24 hr in normal (control) or drug containing RPMI 1640 medium.

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The treatment was done with three concentrations of hexadecylphosphocholine (HPC; obtained from Asta Medica, Bielefeld, FRG): 80 μM, 100 μM and 120 μM for the 2-hr therapy or 40 μM, 60 μM or 80 μM for the 24-hr therapy. Thereafter, the cells were washed twice with normal RPMI 1640 medium. The FDG uptake experiments were performed in triplicate 1 and 4 hr (2-hr therapy) or 1 hr (24-hr therapy) after the end of the therapy. Therefore, the cells were washed twice with phosphate buffered saline (PBS) and incubated in glucose-free RPMI-1640 medium (Gibco BRL) with 292 mg glutamine, 100,000 IE penicillin and 100 mg streptomycin/l at 37° C for 30 min.

Then the uptake experiment was performed with 1 μCi/ml 2-fluoro-2-deoxy-D-(U-^{14}C)-glucose (FDG) (Amersham; specific activity 335 mCi/mmole; radioactive concentration 200 μCi/ml; radiochemical purity 99.3%). An uptake experiment was performed with controls and 80 μM HPC treatment (2 hr incubation in the drug containing medium) with incubation times of 2, 5, 10, 30, 60, 120 and 180 min in the medium containing FDG. The following experiments were done with 60 min incubation. The cells were washed three times with ice-cold PBS after the incubation. Lysis was performed on ice with ice-cold perchloric acid (0.6 M). Thereafter, the lysate was neutralized with 1 M KOH and 0.5 M Tris (pH = 7). Lysates and media were then analyzed with high performance liquid chromatography (HPLC), using the method described by Oberdorfer et al. (*17*).

After continuous detection of radioactivity and differential refractive index, the radioactive peak areas were integrated. The identification of the peaks was done with reference to ^{19}F-NMR spectroscopy of an independently prepared sample and by use of FDG as provided from the manufacturer. The uptake values were normalized to the viable cell number using a cell counter (Coulter Electronics, Dunstable, Beds., UK) and trypan blue staining for the differentiation of viable and dead cells. The FDG uptake was then expressed either as nCi/well or as nCi/10^5 viable cells.

Mono- and Combination Therapy

The cells were trypsinated and seeded in 24-well plates (Greiner). After adhesion they were treated with different schedules (Fig. 1). Single therapy: HPC for 2 hr in concentrations of 80 μM, 100 μM or 120 μM or deoxyglucose (DOG) (Aldrich, Steinheim, FRG) in concentrations of 5 mM and 10 mM in the drug containing medium. Combination therapy: HPC and DOG simultaneously (combination 1 = 5 mM DOG; combination 4 = 10 mM), HPC, 1-hr delay and DOG for 2 hr (combination 2 = 5 mM; combination 2 = 10 mM) or HPC, 4-hr delay and DOG for 2 hr (combination 3 = 5 mM; combination 6 = 10 mM). The cells were counted using a Coulter Counter on Days 1, 2 and 3 after therapy. Four wells were used per day and concentration. Each well was counted three times.

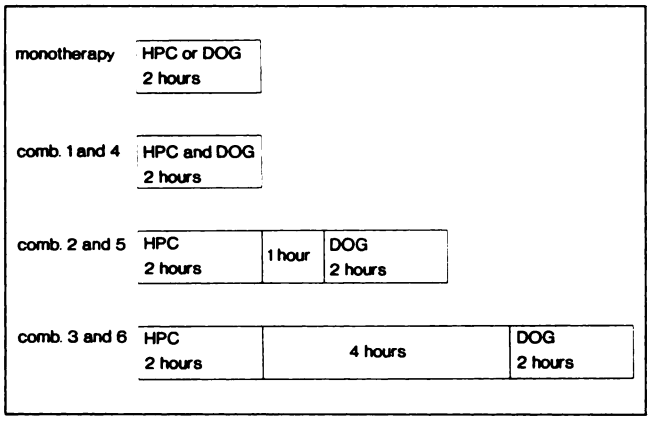

FIGURE 1. Treatment modalities: as monotherapies, three different doses of HPC (80, 100 and 120 μM) and two doses of DOG were used (5 mM and 10 mM). The combination therapy consisted of HPC (80, 100 or 120 μM) and DOG (5 mM or 10 mM), either simultaneously or after a 1-hr or 4-hr delay. The combinations 1, 2 and 3 used 5 mM of DOG and the combinations 4, 5 and 6 used 10 mM of DOG.

RESULTS

Figure 2A shows the time-dependent uptake of FDG during 1 hr for untreated cells and cells 1 hr after a low dose therapy of HPC. The FDG uptake showed a linear increase during the 180-min incubation period. We found a moderate enhancement of the FDG uptake in the treated cells. The difference in FDG uptake between treated and untreated cells was more pronounced for longer incubation times in the FDG-containing medium. In the following experiments, a 60-min incubation period for the measurement of the FDG uptake was chosen.

In Figures 2B and 2C, the effect of three drug concentrations (2-hr therapy) on the FDG uptake is demonstrated. First, a dose dependence was found: the FDG uptake increased with the dose of HPC. Furthermore, a time dependence could be observed: the effects on the FDG uptake were more pronounced 1 hr after therapy than 4 hr after therapy. The normalization of the FDG uptake for the number of viable cells indicated that the increase in FDG uptake was a result of an enhancement of metabolism in the individual tumor cells. More than 92% of the FDG was phosphorylated (FDG-6-phosphate): 92%–98.7% in controls, 95.3%–98% after 80 μM HPC, 96.5%–98.6% after 100 μM HPC and 96.4%–98.8% after 120 μM HPC.

The growth curves for the different doses of HPC as well as the curves for two doses of DOG are demonstrated in Figure 2D. A dose-dependent growth inhibition was found for HPC with its culmination on Day 2 after therapy.

The changes in FDG uptake following a 24-hr therapy are presented in Figure 3. The nCi/well value showed a 25% decrease for 40 μM, a 13% decrease for 60 μM and a 12% decrease for 80 μM (Fig. 3A). However the nCi/10^5 cells value was increased in a dose-dependent manner (Fig. 3B). More than 95% of the radioactivity was FDG-6-phosphate.

The effects of a metabolically designed combination therapy with HPC and DOG in MCF7 cells are seen in Figure 4 and in Table 1. An improvement of growth inhibition was achieved by addition of DOG on Day 1 after therapy for all combinations, as compared to the monotherapy with HPC. However, long-term effects on cell growth were only obtained by the sequential use of HPC and DOG. The improvement of the therapeutic effect was not dependent on the dose of DOG.

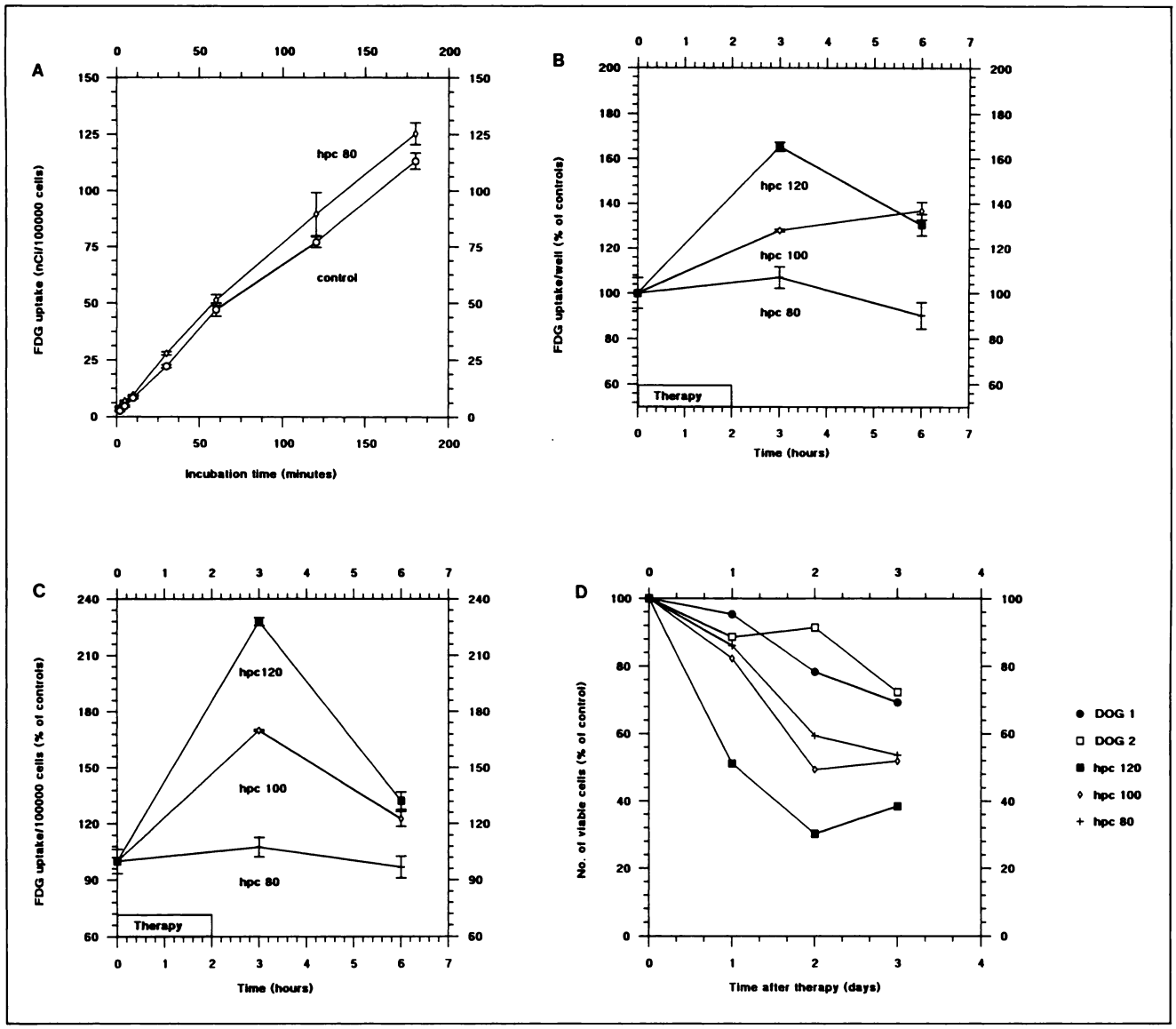

FIGURE 2. (A) FDG uptake (mean values and s.d.) after 2, 5, 10, 30, 60, 120 and 180 min incubation in a ^{14}C-FDG containing medium for treated (80 μM HPC) and untreated (control) cells. The incubation time in the therapy medium was 2 hr. The FDG uptake was determined 1 hr after the end of therapy and is expressed as nCi/10^5 cells. HPLC analysis revealed that more than 92% of the radioactivity in the cells was phosphorylated. (B, C) FDG uptake 1 and 4 hr after 2 hr of incubation in the drug containing medium. HPC was applied in concentrations of 80, 100 and 120 μM. The FDG uptake values after 60 incubation in the glucose free medium is expressed as nCi/well (Fig. 2B) or as nCi/10^5 cells (Fig. 2C) and percent of the control. More than 92% of the radioactivity was phosphorylated as determined by HPLC analysis. (D) Results of monotherapy (median values; n = 12): HPC was applied in concentrations of 80, 100 and 120 μM. The DOG treatment was performed either with 5 mM or 10 mM. The viable cell number was determined on Days 1, 2 and 3 after the end of therapy and expressed as a percent of the control.

DISCUSSION

In chick fibroblasts it has been shown that DOG is metabolized to the 6-phosphate derivative, the 1-phosphate derivative, the 1,6-biphosphate derivative, the UDP- and GDP-derivative and to 2-deoxy-D-gluconic acid 6-phosphate (*18*). Using HPLC analysis and FDG, we found that 95% were present in its 6-phosphate derivative. Other metabolites than FDG and FDG-6-phosphate were not detected. Their concentrations may be below the detectable level in the present assay. Furthermore, Minn et al. report similar results in Lewis lung tumors: in their study most of the radioactivity (85%–90%) was found as FDG-6-phosphate (*14*). Moreover differences in the kinetics of the DOG or FDG metabolism, differences in the cell system (chick fibroblasts versus mammary carcinoma cells) or differences in the deproteination methods may account for the fact that no metabolites other than FDG and FDG-6-phosphate were detected.

We found a dose-dependent increase in FDG uptake

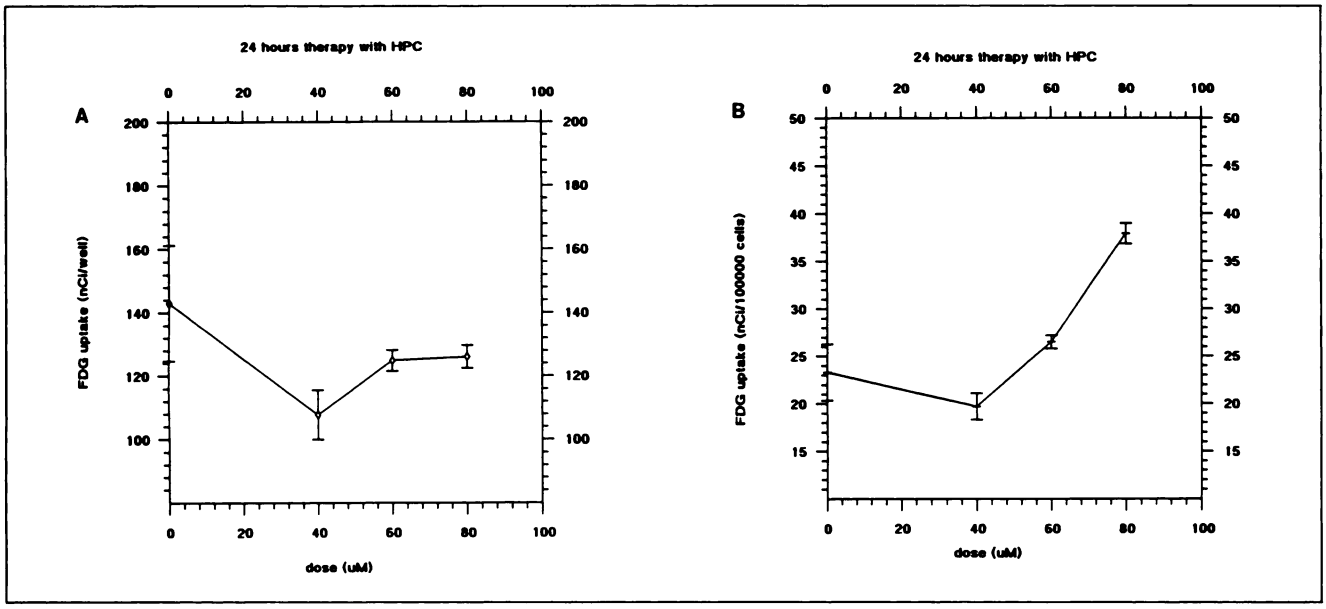

FIGURE 3. FDG uptake (mean values and s.d.) 1 hr after 24-hr of incubation in the drug containing medium. HPC was applied in concentrations of 40 μM, 60 μM and 80 μM. The uptake is expressed as nCi/well (Fig. 3A) or as nCi/10^5 cells (Fig. 3B). The phosphorylated fraction of the accumulated radioactivity was 95%–96%.

after the 2-hr therapy for both the nCi/well value and the nCi/10^5 cells value. After the 24-hr therapy the nCi/well value showed a decrease, whereas the nCi/10^5 cells value increased in a dose-dependent manner. The nCi/well values show that the measurement of the FDG uptake in a given volume may decrease after therapy. However when the radioactivity was related to the viable cell number, an increase was observed, indicating that the metabolism of the individual tumor cell was enhanced. If the uptake/well is interpreted as a model for the tumor in vivo, then it seems that the FDG uptake decreases, due to a lower number of viable tumor cells in a treated lesion. This rather trivial finding is accompanied by a finding with possible clinical impact: the resting cells in the tumor may show an enhancement of the FDG uptake, which may be used for specific modifications of a given therapy.

Überall et al. found that cells treated with HPC exhibit normal levels of cytosolic Ca^{2+} and suggested that this gives strong evidence against extensive membrane disintegration or leakage as a consequence of HPC treatment (*8*). This observation, together with the fact that most of the radioactivity in our study was phosphorylated, indicate that the effects on FDG uptake were not caused simply by membrane leakage.

The increase in FDG uptake shows that the tumor metabolism is an important factor in the response of the tumor cells to a specific therapy. In patients with advanced breast cancer, Minn et al. observed that an increased FDG uptake after therapy was associated with clinical progression (*12*), whereas a decreased uptake of FDG was associated with tumor regression (*15*). They hypothesized that the FDG uptake was related to the viability of tumor cells. Our data show that the FDG uptake after a given therapy is not only a function of the number of tumor cells, but also dependent on the metabolic state of the individual tumor cell.

Using Lewis lung carcinoma, Minn et al. observed that doxorubicin seemed to enhance the FDG uptake and to raise the proportion of FDG-6-phosphate (*14*). Lyon et al. found an enhancement of glycolysis in Adriamycin-resistant MCF7 cells, which was present even in the absence of drugs (*19*). Similar results were obtained by Kaplan et al., who noticed that resistant MCF7 cells accumulated deoxyglucose-6-phosphate faster and to a greater extent than their sensitive parent line (*20,21*). The increase of glucose metabolism may be necessary for the energy requirements of the drug efflux and the detoxification processes.

These metabolic changes are not only seen in chemotherapy, but are also observed in radiation therapy. Jain et al. found that glycolysis is linked to DNA repair (*22*), as well as to the repair of potentially lethal damage (*23*). In our experiments, the reaction of the tumor cells to HPC was a dose-dependent increase of the glucose metabolism, as indicated by an enhancement of the FDG uptake. This finding may be explained by the energy requirements of drug transport and detoxification processes in surviving cells.

Interestingly, animal experiments revealed that a complete disappearance of macroscopic tumors during HPC therapy was followed by a recurrence of the tumors shortly after cessation of therapy (*4*). Moreover, recent studies relate drug resistance to glucose metabolism. Vera et al. (*24*) observed that in Xenopus laevis oocytes, which had been injected with RNA encoding the rat erythroid glucose transporter (GLUT1) and expressing the corresponding protein, the accumulation of ^{3}H-vinblastin was decreased.

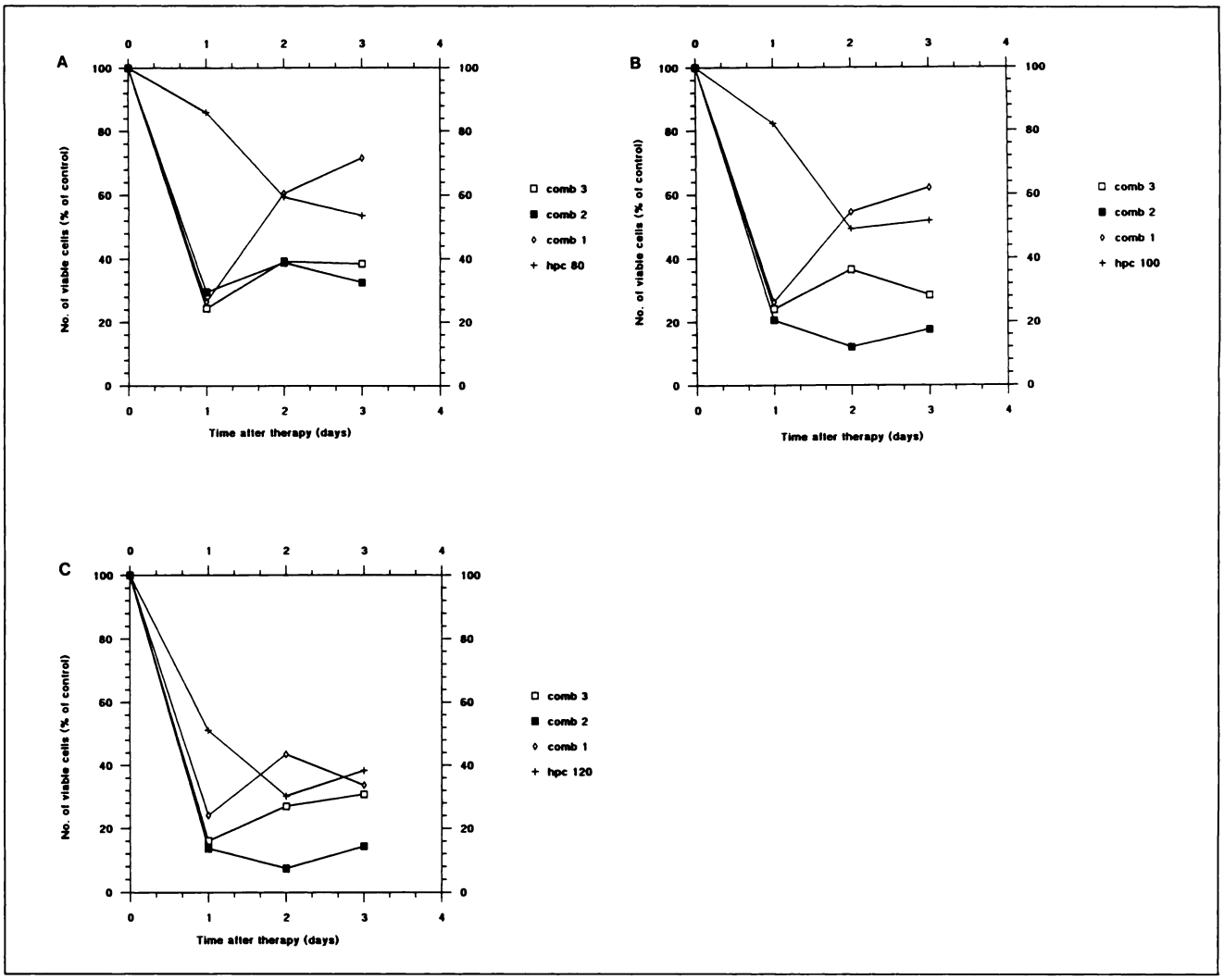

FIGURE 4. Results of HPC monotherapy and different combination therapies (median values; n = 12). The viable cell number was determined on Days 1, 2 and 3 after the end of therapy and are expressed as a percent of the control. Combinations were used with 80 μM HPC (A), 100 μM HPC (B) and 120 μM HPC (C). See also Table 1.

Moreover, the efflux of the drug out of the cell increased. These effects were inhibited by addition of cytochalasin B and phloretin, two inhibitors of glucose transporters. However, the mechanism by which the GLUT1 may contribute to the drug resistance is not clear.

Evidence for the energy-dependence of drug resistance is reported by Versantvoort et al. (*25*). In multidrug-resistant human lung cancer cell lines without glycoprotein P expression, the reduced accumulation of daunorubicin could be reversed by cellular energy depletion. The authors suggested that the drug accumulation in these cells was reduced by an energy-dependent drug efflux system.

However, the increase of the glucose metabolism may be a general feature of stressed cells. Pasternak et al. found a stimulation of the lactate production as well as an increase of the ^{3}H-deoxyglucose uptake in BHK-21 cells after transfection with Semliki Forest virus or exposure to arsenite or azide (*26*). They suggested that the stimulation of the glucose metabolism is at least in part controlled at the level of glucose transport. Widnell et al. observed that a stress induced increase in DOG uptake can be explained by a redistribution of the glucose transport protein from a perinuclear region to the plasma membrane (*27*). Moreover, the stimulation of the DOG uptake in BHK cells was reversible with $T_{1/2}$ of 1–2 hr (*26*). This agrees with our finding of a short-term increase of the FDG uptake in treated MCF7 cells.

The next step of our approach exploited the metabolic reaction observed in the FDG uptake experiments. Our goal was to disturb possible repair processes using DOG (Fig. 1), which is known to have an antitumor effect (*28–32*). In studies with rat brain homogenates, Horton et al. observed a 40% inhibition of hexokinase by DOG, not by deoxyglucose-6-phosphate, whereas hexose-phosphate-isomerase was 70% inhibited, and phosphofructokinase was 17% inhibited, by deoxyglucose-6-phosphate (*33*). They supposed two mechanisms of action: First, a competitive inhibition of glucose transport into the brain; later, a

TABLE 1
Results of Mono- and Combination Therapy: Viable Cell Number on Days 1, 2 and 3 After Therapy

Therapy type	Day 1	Day 2	Day 3
control	442.8 ± 83.9	917.9 ± 183.9	1741.6 ± 298.2
DOG 5 m*M*	366.4 ± 42.4	697 ± 123	1363 ± 274.7
DOG 10 m*M*	399.9 ± 39.1	624.2 ± 52.4	1349 ± 88.3
HPC80	382.3 ± 57.5	483.6 ± 56.8	1069.4 ± 139.9
comb 1	116.9 ± 44.8	463 ± 91.6	1292.1 ± 274
comb 2	128 ± 11.9	287.8 ± 67.3	633.5 ± 57.5
comb 3	120.3 ± 47.4	316.5 ± 18	805 ± 271.2
comb 4	302.4 ± 78.4	756.9 ± 95.6	1075.6 ± 97.1
comb 5	104.9 ± 16.4	219.7 ± 39	693.3 ± 139.6
comb 6	134.9 ± 35	447.9 ± 148.5	756.3 ± 143.5
HPC 100	353 ± 31.5	389.9 ± 61.7	985.3 ± 194.6
comb 1	134.8 ± 50.1	441.3 ± 26	1191.8 ± 104.6
comb 2	91.1 ± 13.7	102.5 ± 24.8	350.5 ± 139.2
comb 3	112.7 ± 44.7	292.2 ± 43.2	599.3 ± 175.2
comb 4	201.5 ± 46.5	594.9 ± 116.7	983.3 ± 126.2
comb 5	106.3 ± 23.2	142 ± 38.2	516 ± 143.2
comb 6	102.8 ± 18.6	361.5 ± 71.9	715.4 ± 157.8
HPC 120	228.3 ± 42.7	242.6 ± 116.8	766.3 ± 218
comb 1	100.3 ± 15.6	360.3 ± 70.4	621.4 ± 103.8
comb 2	58.6 ± 9.2	68.2 ± 23	300.4 ± 55
comb 3	68.9 ± 13.1	232.8 ± 26.4	667 ± 358.9
comb 4	222.3 ± 86.9	422.3 ± 68.1	1108.8 ± 276
comb 5	75.5 ± 16.7	115.7 ± 42.1	319.1 ± 34.6
comb 6	101.8 ± 21.9	304.3 ± 52.1	804.2 ± 202.1

Mean values and standard deviations (n = 12). DOG = deoxyglucose; HPC 80 = 80 μ*M* hexadecylphosphocholine (HPC); HPC 100 = 100 μ*M* HPC; HPC 120 = 120 μ*M* HPC.

stronger inhibition of glycolysis by the high concentration of deoxyglucose-6-phosphate and the consecutive inhibition of the activity of isomerase and other glycolytic enzymes.

In mammary tumor cells and hepatoma cells, DOG, FDG and other sugar analogs caused a depletion of the UTP pools and often also of the CTP pools (*34,35*). In s.c. methylcholanthrene-induced sarcomas, DOG resulted in a decreased ATP to inorganic phosphate ratio, as measured in vivo with ^{31}P magnetic resonance spectroscopy (*36*). Furthermore, Kaplan et al. (*19*) observed a decrease of ATP and phosphocreatine content in MCF7 cells after exposure to DOG. This decrease, as well as the growth inhibition, was stronger in drug resistant cells than in the sensitive parent line.

This rather selective toxicity of DOG for resistance cells directs attention to a link between glucose metabolism and drug resistance. Therefore, DOG and other sugar analogs may be used in combination treatment to achieve a higher cytostatic effect if drug resistance is present. This hypothesis is supported by results from Jain et al. (*22,23*), who described an inhibition of DNA repair and fixation of cell damage by DOG, which seems to be a result of energy-linked inhibitions of repair mechanisms.

HPC's action may be twofold: the drug itself may have a cytotoxic or cytostatic capacity, and in addition, cytotoxic metabolites may have a second and later effect on the tumor cells (*4,5*). DOG, given simultaneously with HPC, may prevent the production of these metabolites and, therefore, inhibit the effects on future cell growth. This could explain why the simultaneous therapy with HPC and DOG yielded no better—or even worse—results than those from monotherapy with HPC on Days 2 and 3 after therapy.

The best results were obtained with the combinations during the period of highest FDG uptake. One hour after HPC therapy, the tumor cells may show increased energy requirements for the detoxification, causing an enhanced uptake of sugars or other fuels. In our study, we found an increased uptake of FDG. This can be used to transport a toxic sugar (DOG) into the cell, which has its own effect on the tumor cell and disturbs the detoxification mechanisms in response to the chemotherapeutic agent. An interesting finding is that the dose of DOG had no major effect: no difference was observed between 5 m*M* and 10 m*M* of DOG. This indicates that the main mechanism of action may be the disturbance of the detoxification processes.

The synergistic action of DOG was observed for all doses of HPC. Moreover, we found that the lowest dose of HPC, together with DOG, yielded a better result than the highest monotherapy with HPC. Therefore, this combination can be used to achieve a good therapeutic outcome together with low side effects. However, to exclude a synergistic effect on normal tissues that may cause increased side effects, this schedule has to be tested in nontumor cells.

As demonstrated by these experiments, FDG metabolism can be used to develop a therapy directed against processes that are in need of energy. Whatever the difference in metabolism between FDG and glucose, the enhanced FDG uptake indicates that there is an enhancement of glucose metabolism. This enhancement can be used for a metabolic approach to combination therapy. The design of such a treatment requires data about the changes in metabolic pathways and the key enzymes of these pathways, as well as the time and dose dependence of these changes.

PET seems to be an appropriate method to gain such data. However, one has to keep in mind that a considerable amount of the tumor mass consists of normal cells, as fibroblasts, lymphocytes and macrophages etc. It has been observed that human breast cancers contain a variable but substantial amount (19%–64%) of macrophages (*37*), whereas the lymphocyte fraction may reach 10% (*38*).

These nontumor fractions may cause problems for the assessment of the tumor metabolism prior to therapy because they contribute to the signal being measured; this has been shown in a NMR spectroscopy study (*39*). During or after therapy, the amount of macrophages or other immune cells may increase due to the presence of cell detritus. Preliminary data in rats bearing methylnitrosou-

rea induced mammary carcinomas and treated with HPC show that the FDG uptake decreased in only 30% of these tumors (*40*). The finding that there is no change in the other tumors may be explained by a loss of tumor cells, together with an enhancement of the FDG uptake in the resting cells. However, more in vivo studies are needed to rule out a possible contribution of nontumor fractions to the signal being measured with PET.

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REFERENCES

1. Weber G. Enzymology of cancer cells. Part 1. *N Engl J Med* 1977;296: 486–493.
2. Weber G. Enzymology of cancer cells. Part 2. *N Engl J Med* 1977;296: 541–551.
3. Weber G. Biochemical strategy of cancer cells and the design of chemotherapy: G.H.A. Clowes Memorial lecture. *Cancer Res* 1983;43: 3466–3492.
4. Berger MR, Muschiol C, Schmähl D, Eibl HJ. New cytostatics with experimentally different toxic profiles. *Cancer Treatment Rev* 1987;14: 307–317.
5. Hilgard P, Stekar J, Voegeli R, et al. Characterization of the antitumor activity of hexadecylphosphocholine (D 18506). *Eur J Cancer Clin Oncol* 1988;21:1457–1461.
6. Hochhuth C, Berkovic D, Eibl HJ, Unger C, Doenecke D. Effects of antineoplastic phospholipids on parameters of cell differentiation in U937 cells. *Eur J Cancer Res Clin Oncol* 1990;116:459–466.
7. Fleer AM, Unger C, Kim DJ, Eibl H. Metabolism of ether phospholipids and analogs in neoplastic cells. *Lipids* 1987;22:856–881.
8. Überall F, Oberbhuber H, Maly K, Zaknun J, Demuth L, Grunicke HH. Hexadecylphosphocholine inhibits inositol phosphate formation and protein kinase C activity. *Cancer Res* 1991;51:807–812.
9. Haberkorn U, Strauss LG, Dimitrakopoulou A, et al. PET studies of fluorodeoxyglucose metabolism in patients with recurrent colorectal tumors receiving radiotherapy. *J Nucl Med* 1991;32:1485–1490.
10. Abe Y, Matsuzawa T, Fujiwara T, et al. Assessment of radiotherapeutic effects on experimental tumors using ^{18}F-2-fluoro-2-deoxy-D-glucose. *Eur J Nucl Med* 1986;12:325–328.
11. Kangas L, Paul R, Kellokumpu-Lehtinen P, Harju-Jeanty R, Tuominen J. Rats with mammary cancer treated with toremifene and interferon: morphometry and needle aspiration biopsy for determination of ATP and ^{14}C-fluorodeoxyglucose content. *Res Exp Med* 1989;189:113–119.
12. Minn H, Soini I. (^{18}F)Fluorodeoxyglucose scintigraphy in diagnosis and follow up of treatment in advanced breast cancer. *Eur J Nucl Med* 1989; 15:61–66.
13. Minn H, Paul R, Ahonen A. Evaluation of treatment response to radiotherapy in head and neck cancer with fluorine-18 fluorodeoxyglucose. *J Nucl Med* 1988;29:1521–1525.
14. Minn H, Kangas L, Kellokumpu-Lehtinen P, et al. Uptake of 2-fluoro-2-deoxy-D-(U-^{14}C)-glucose during chemotherapy in murine Lewis lung tumor. *Nucl Med Biol* 1992;19:55–63.
15. Kubota K, Ishiwata K, Kubota R, et al. Tracer feasibility for monitoring tumor radiotherapy: a quadruple tracer study with fluorine-18-fluorodeoxyglucose or fluorine-18-fluorinedeoxyuridine, L-(methyl-^{14}C)methionine, (6-^{3}H)thymidine, and gallium-67. *J Nucl Med* 1991;32:2118–2123.
16. Wahl RL, Cody RL, Hutchins CD, Mudgett EE. Primary and metastatic breast carcinoma: initial clinical evaluation with PET with the radiolabeled glucose analogue 2-(F-18)-fluoro-2-deoxy-D-glucose. *Radiology* 1991;179: 765–770.
17. Oberdorfer F, Kemper J, Kaleja M, Reusch J, Gottschall K. Carbohydrate analyses with ion chromatography using eurokat stationary phases. Preparative separation of monosaccharides and their fuorinated derivatives. *J Chrom* 1991;522:483–487.
18. Schmidt MFG, Schwarz RT, Scholtissek C. Nucleotide diphosphate derivatives of 2-deoxy-D-glucose in animal cells. *Eur J Biochem* 1974;49: 237–247.
19. Lyon RS, Cohen JS, Faustino PJ, Megnin F, Myers CE. Glucose metabolism in drug-sensitive and drug-resistant human breast cancer cells monitored by magnetic resonance spectroscopy. *Cancer Res* 1988;48:870–877.
20. Kaplan O, Navon G, Lyon RC, Faustino PJ, Straka EJ, Cohen JS. Effects of 2-deoxyglucose on drug-sensitive and drug-resistant human breast cancer cells: toxicity and magnetic resonance spectroscopy studies of metabolism. *Cancer Res* 1990;50:544–551.
21. Kaplan O, Jaroszewski JW, Clarke R, et al. The multidrug resistance phenotype: ^{31}P nuclear magnetic resonance characterization and 2-deoxyglucose toxicity. *Cancer Res* 1991;51:1638–1644.
22. Jain VK, Kalia VK, Sharma R, Maharajan V, Menon M. Effects of 2-deoxy-D-glucose on glycolysis, proliferation kinetics and radiation response of human cancer cells. *Int J Radiat Oncol Biol Phys* 1985;11:943–950.
23. Jain VK, Gupta J, Lata K. Energetics of cellular repair processes in a respiratory deficient mutant of yeast. *Radiat Res* 1982;92:463–473.
24. Vera JC, Castillo GR, Rosen OM. A possible role for a mammalian facilitative hexose transporter in the development of resistance to drugs. *Mol Cell Biol* 1991;11:3407–3418.
25. Versantvoort CHM, Broxterman HJ, Pinedo HM, et al. Energy-dependent processes involved in reduced drug accumulation in multidrug-resistant human lung cancer cell lines without P-glycoprotein expression. *Cancer Res* 1992;52:17–23.
26. Pasternak CA, Aiyathurai JEJ, Makinde V, et al. Regulation of glucose uptake by stressed cells. *J Cell Physiol* 1991;149:324–331.
27. Widnell CC, Baldwin SA, Davies A, Martin S, Pasternak CA. Cellular stress induces a redistribution of the glucose transporter. *FASEB J* 1990; 4:1634–1637.
28. Laszlo J, Humphreys SR, Goldin A. Effects of glucose analogues on experimental tumors. *J Natl Cancer Inst* 1960;24:267–281.
29. Ball HA, Wick AN, Sanders C. Influence of glucose antimetabolites on the Walker tumor. *Cancer Res* 1956;17:235–239.
30. Sokoloff HB, Eddy WH, Saelhoff CC, Beach J. Glucose antagonists in experimental cancer. *Arch Pathol Lab Med* 1955;59:729–732.
31. Woodward GE, Hudson MT. The effect of 2-desoxy-D-glucose on glycolysis and respiration of tumor and normal tissues. *Cancer Res* 1954;14: 599–605.
32. Bessell EM, Courtenay VD, Foster AB, Jones M, Westwood JH. Some in vivo and in vitro antitumor effects of the deoxyfluoro-D-glucopyranoses. *Europ J Cancer* 1973;9:463–470.
33. Horton RW, Meldrum BS, Bachelard HS. Enzymic and cerebral metabolic effects of 2-deoxy-D-glucose. *J Neurochem* 1973;21:507–520.
34. Holstege A, Schulz-Holstege C, Henninger H, Reiffen KA, Schneider F, Keppler D. Uridylate trapping induced by the C-2-modified D-glucose analogs glucosone, fluoroglucose, and glucosamine. *Eur J Bioch* 1982; 121:469–475.
35. Keppler D, Fauler J, Gasser T, et al. Uridylate-trapping sugar analogs in combination with inhibitors of uridylate synthesis de novo and 5-fluorouridine. *Adv Enzyme Regul* 1985;23:61–79.
36. Karczmar GS, Arbeit JM, Toy BJ, Speder A, Weiner MW. Selective depletion of tumor ATP by 2-deoxyglucose and insulin, detected by ^{31}P magnetic resonance spectroscopy. *Cancer Res* 1992;52:71–76.
37. Steele RJC, Brown M, Eremin O. Characterization of macrophages infiltrating human mammary carcinomas. *Br J Cancer* 1985;51:135–138.
38. Whitford P, Mallon EA, George WD, Campbell AM. Flow cytometric analysis of tumor infiltrating lymphocytes in breast cancer. *Br J Cancer* 1990;62:971–975.
39. Smith TAD, Glaholm J, Leach MO, Machin L, McCready VR. The effect of intra-tumor heterogeneity on the distribution of phosphorus-containing metabolites within human breast tumors: an in vitro study using ^{31}P NMR spectroscopy. *NMR in Biomed* 1991;4:262–267.
40. Haberkorn U, Strauss LG, Ziegler S, et al. (^{18}F)Fluorodeoxyglucose uptake for the measurement of metabolic therapy effects in a mammary carcinoma model. In: Breit A, ed. *Tumor response monitoring and treatment planning.* Berlin, Heidelberg: Springer; 1992:183–187.