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Insulin dependency of F-18-fluorodeoxyglucose accumulation in breast carcinoma cells compared to Tl-201 uptake

Heike Wolf, Winfried Brenner, Volker Stein, Stephan Tinnemeyer, Karl Heinz Bohuslavizki, Ulrich Teichert, Malte Clausen, Eberhard Henze

Clinic of Nuclear Medicine, Christian-Albrechts-University of Kiel, Kiel, Germany

The effect of euglycemic hyperinsulinism on ^{18}F-fluorodeoxyglucose (FDG) uptake in cultures of breast cancer was determined, in comparison to ^{201}Tl. Measurements of both tracers were performed on 168 cell culture tubes, with incubation intervals ranging from 1 to 240 min. Linear accumulation of FDG over time was observed both with and without insulin. A significant increase from 3.52 ± 0.74 % to 5.10 ± 0.32 % over 240 min was attained after adding insulin. In contrast, ^{201}Tl revealed only a slightly significant increase after insulin. By extrapolating these results to FDG PET tumor imaging, a markedly improved tumor targeting might be obtained by providing a state of euglycemic hyperinsulinism, i.e. replacing the commonly used single FDG injection by a continuous FDG/glucose/insulin infusion. An optimum imaging period of 150 min after starting the infusion can be derived from our data, considering the decay of ^{18}F.

Key words: breast neoplasms; tumor cells, cultured; euglycemic hyperinsulinism; deoxyglucose, ^{18}F-fluorodeoxyglucose; thallium radiosiotopes, ^{201}Tl

Introduction

Increased glycolysis is an important characteristic of cancer cells.[1, 2] Positron emission tomography (PET) with [^{18}F]-2-fluoro-2-deoxy-D-glucose (FDG) is used as a suitable indicator of the glycolytic activity of tumors. FDG is rapidly transported into the tumor cells and phosphorylated by hexokinase to FDG-6-phosphate, but is not further metabolized,[3, 4] meanwhile the physiologic substrate glucose enters the glycolytic pathway. Increased FDG uptake imaged by PET has been reported in many types of human tumors, e.g., head and neck cancer,[5] lung,[6] colon,[7, 8] liver[9, 10] and breast cancer.[11, 12]

^{201}Tl SPECT imaging might work as well as FDG PET in the detection of viable tumor tissue based on the relatively enhanced tumor blood supply. In neoplastic cell cultures the ^{201}Tl uptake increases in conjunction with the cell's metabolic activity, thereby confirming that it might also reflect tumor growth rather than just tumor perfusion.[13]

In the in-vivo and in-vitro studies so far available[5, 12, 14–19] controversial effects of plasma insulin and/or glucose levels on FDG tumor targeting have been reported. Agreement consists in most studies that elevated cold glucose levels seem to compete with FDG, thus hampering FDG accumulation.

The purpose of this study was to determine the effect of euglycemic hyperinsulinism on the cellular uptake of FDG in cultures of breast cancer cells, and to compare it with the uptake of ^{201}Tl under identical conditions, since insulin effects on ^{201}Tl tumor accumulation are also hardly known.

Correspondence to: Heike Wolf, Ph. D. Christian-Albrechts-University of Kiel, Clinic of Nuclear Medicine, Arnold-Heller-Str. 9, D-24105 Kiel, Germany

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Materials and methods

Radiopharmaceuticals

FDG (BTZ Beschleuniger- und Tomographiezentrum Hamburg, Germany) was obtained with a radiochemical purity higher than 98 %. Impurities like ethanol, acetonitrile and acetone were less than

0.06 mg/ml and the glucose concentration was less than 0.6 mg/ml.

A commercially available ^{201}Tl thallous chloride was used (Mallinckrodt, Hennef, Germany) as a reference tracer.

Cell cultivation

The human breast carcinoma cell line (MCF 7, dkfz – Tumorbank, Heidelberg, Germany) chosen for examination was maintained in a medium containing 5 mmol/l glucose, supplemented with 10 % fetal calf serum (Boehringer Mannheim, Mannheim, Germany), 0.5 % L-glutamine 20 mmol/l (Biochrom, Berlin, Germany) and 1ml gentamycin 0.1 mg/ml (Merck, Darmstadt, Germany). The cell line grew well in vitro as a monolayer and had a doubling time of approximately 40 h. A Fuchs-Rosenthal counting chamber was used for cell counting and the trypan blue exclusion method for viability determination was conducted using an inverted Leitz microscope.

The total number of cells ranged from 7.9 to 9.2 million cells per tube with a median of 8.5 million. Cell viability was higher than 90 %.

Uptake measurements

30 MBq FDG and 3 MBq ^{201}Tl were added to 250 ml medium, thus maintaining a continuous supply during the whole incubation period. For each tube, 3 ml medium was used, and the exponentially growing cells were incubated with FDG and ^{201}Tl at 37 °C. Influx was stopped at different incubation intervals ranging from 1 minute to 4 hours, by removing the medium. Subsequently, cells were washed rapidly 3 times with 4 °C saline solution for a total of 15 seconds and harvested with trypsin-EDTA (Sigma Chemie, Deisenhofen, Germany).

The radioactivity was measured with a gamma well counter in a definite geometry. Total activity per culutre tube was attained by measuring the tube before removing the medium. The cellular uptake was determined after the washing phase. FDG measurements were performed first and samples of medium and cells were stored for two days to determine the ^{201}Tl uptake, after the complete decay of ^{18}F. All results were corrected for physical decay.

To assure adequate measurement statistics, six tubes per exposure period were used, totaling 84 culture tubes in all. The experiment was repeated exactly under the same conditions, but with an additional administration of insulin, in a quantity of 0.5 IU to the medium, i.e. 2 mIU/ml, providing a complete receptor saturation.

Uptake results are presented as the percentage of the activity accumulated within the cells, related to the activity added in each tube normalized to 1 million cells, and expressed as mean ± 1 s.d. For statistical comparison, Student's t-test for unpaired data was used.

Results

As shown in Figure 1, linear accumulation of FDG over time was observed in nearly all the exposures, up to a maximum of 240 min. With the longest exposure period of 240 min an uptake of 3.52 ± 0.74 % was measured under baseline conditions. A further significant increase of up to 5.10 ± 0.32 % was attained after adding insulin, with $p \leq 0.02$ already beginning after an incubation time of 20 min.

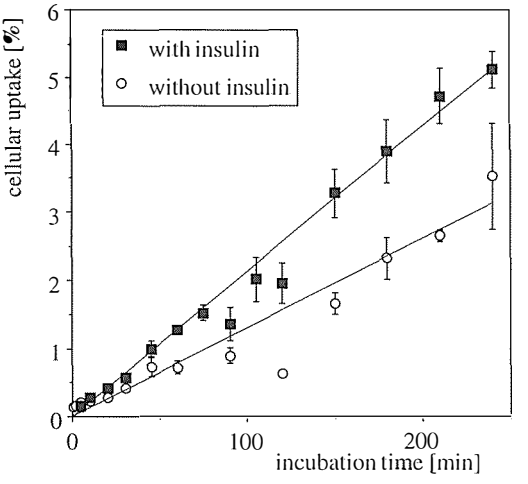

Figure 1. Cellular uptake of ^{18}FDG expressed as % of medium activity per 1 million breast carcinoma cells with and without insulin (0.5 mIU/ml medium) at various incubation time intervals. Results are given as mean of 6 culture tubes ± 1 SD. Additional insulin: filled squares; without insulin: open circles.

In contrast, ^{201}Tl accumulation occured at a high rate during the first 10 to 20 min and then reached a plateau. Adding insulin did not induce such marked effects, as illustrated in Figure 2.

By lumping all the tubes, with and without insulin, together, the groups differ with 0.33 ± 0.06 % and 0.25 ± 0.05 %, respectively. Significant differences with $p < 0.02$ might be obtained in the initial phase, such as at 10, 30, 45 or 90 min, while after a 150 min exposure time, both curves converge on each other.

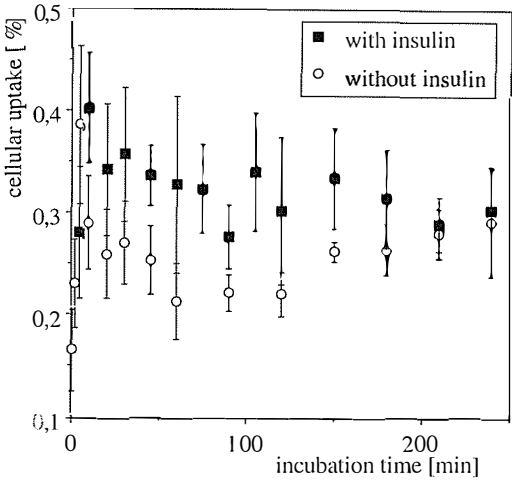

Figure 2. Cellular uptake of ^{201}Tl expressed as % of medium activity per 1 million breast carcinoma cells with and without insulin (0,5 mIU/ml medium) at various incubation time intervals. Results are given as mean of 6 culture tubes ± 1 SD. Additional insulin: filled squares; without insulin: open circles.

Discussion

Tumor targeting and quantitative evaluation of tumor tissue viability with FDG and PET have shown encouraging results, with high tumor/background ratios. Attempts have been made to further increase FDG accumulation in tumor tissue for even better scintigraphic tumor delineation, mainly by using additional glucose administration on induce a state of hyperglycemic hyperinsulinism.[14–17, 20] In agreement with in-vitro studies, examing the same issue, FDG accumulation mainly decreased with elevated plasma or media glucose concentrations[12, 15, 21] except in brain tumor imaging.[17] This was interpreted as a competing effect between cold and labeled glucose leading to the conclusion that FDG tumor imaging ought to be performed under conditions of food abstinence.[14, 15, 18]

The purpose and the results of this study have to be seen in that context. Because of the obviously hampering FDG accumulation at increased plasma glucose levels, the approach was different to all previously performed studies. The experimental design simulated a state of euglycemic hyperinsulinism in conjunction with a relatively constant supply of FDG, maintained for up to 240 min. Providing this environment to breast cancer cells a significant increase of FDG uptake in tumor tissue was observed as seen in Figure 1. This result seems to support the above cited hypothesis of competition between cold and labeled glucose.

The effect of the same study design on ^{201}Tl cell accumulation was considerably less, and completely concomitant to recent results.[19] Particularly during the initial exposure period of up to 150 min, however, insulin increased the ^{201}Tl uptake by 50 %. Provided this also holds true for in vivo scintigraphy, an increase of the tumor/background ratio by a factor of 1.5 would still represent a great progress in scintigraphic tumor targeting.

Based on this data it seems thus justified to propose a modified application protocol for both ^{201}Tl and FDG tumor imaging, suggesting euglycemic hyperinsulinism and maintaining constant tracer supply for a longer period of time. In the case of ^{201}Tl, an approximately 60 min infusion might be suggested, which causes no additional problem, since insulin clamping requires a continuous infusion anyway. In the case of FDG, the short tracer half life has to be considered. As seen in Figure 1 constant tracer supply results in linear accumulation, if corrected for physical decay. Considering ^{18}F decay an accumulation type of curve is obtained, as shown in Figure 3, with maximum tracer concentration and, thus, a postulated optimum for imaging, at approximately 150 min after starting the FDG/insulin infusion. The dose of insulin should be moderate, i.e. 20–30 IU/h, so as not to induce significant hypoglycemia and/or clinical symptoms, but to raise the plasma insulin level.

Clinical trials are needed to validate this postulated approach. The outcome of such trials is difficult to predict, since the effect of euglycemic hyper-

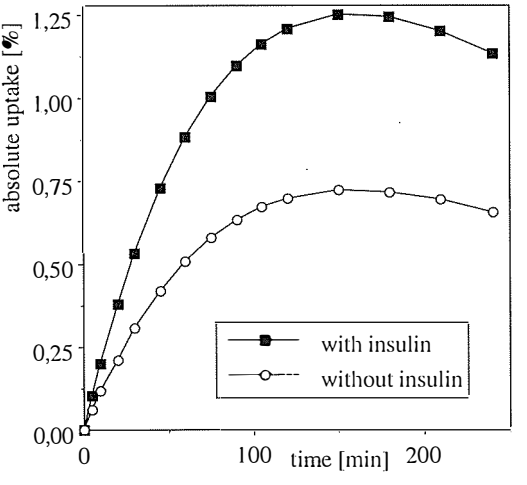

Figure 3. Cellular uptake of ^{18}FDG with (filled squares) and without (open circles) insulin considering physical decay of ^{18}F, as calculated from the regression curves of Figure 1.

insulinism on both the tumor and the surrounding tissue in vivo is uncertain because of the various distribution of the, at least five different, glucose transporting molecules, and the unknown magnitude as to whether these glucose transporters are involved in the facilitated transmembraneous glycolytic flux.[12, 14–18]

Conclusion

The accumulation of FDG and, to a lesser extent, that of ^{201}Tl, in breast carcinoma cells can be significantly increased by insulin and euglycemia. The potential improvement of PET and SPECT tumor targeting ought to justify further clinical testing.

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