Human Melanoma Metabolic Network Analysis with Combined 13C NMR/Bioreactor Technique: Testing the Warburg Effect

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Introduction

The bioreactor techniques are becoming an important tool to study cancer cell metabolism (1). Modeling of intracellular MRS isotopomer data obtained during perfusion with ^{13}C labeled substrates allows quantitative determination of metabolic fluxes *in vivo/in situ*. To our knowledge, no ^{13}C metabolic model exists to determine fluxes from non-steady state data for proliferating cells. Dynamic isotopomer modeling takes optimal advantage of biochemical information that can be assessed with NMR spectroscopy by considering all the different isotopomers of metabolites. The goal of the present work was to develop a ^{13}C metabolic "bonded cumomer" (2) modeling approach adapted for fitting Continuous-Stirred Tank (CST) Bioreactor data obtained with ^{13}C glucose as substrate and to calculate how much energy comes from aerobic glycolysis (Warburg effect) vs oxidative phosphorylation.

Methods

Human DB1 melanoma cells were grown in culture on nonporous polystyrene beads and loaded into a bioreactor consisting of an NMR tube together with porous collagen beads (1). The cells were perfused with 26 mM [1,6-$^{13}C_2$] glucose at 37^0C while the ^{13}C NMR spectrum was recorded with a 9.4 T/89 mm vertical bore Varian Spectrometer. The time courses of [3-^{13}C] lactic acid, [4–^{13}C]-, [3–^{13}C]- and [2–^{13}C] glutamate as well as the glutamate-C4d34 doublet were monitored. Data were analyzed with a metabolic network model that included glucose and lactate transport, glycolysis, TCA cycle, pentose-phosphate pathway, α-ketoglutarate-glutamate and oxaloacetate-aspartate exchange, pyruvate carboxylase activity, anaplerosis at the succinyl-CoA level, pyruvate recycling through malic enzyme and lactate dehydrogenase activity. Isotope balance equations were derived for every bonded cumomers of orders 1,2,3 of glutamate, glutamine and aspartate. This resulted in a set of ~150 differential equations that were solved to determine flux through the various metabolic pathways by isotopomer/cumomer analysis (2). Solving a system of differential equations (using the Runge-Kutta algorithm) in terms of bounded cumomers yields time courses for all possible fine structure ^{13}C multiplets of e.g glutamate, glutamine and aspartate. Minimization was performed using BFGS or Simplex algorithms. Proper mean-square convergence was confirmed by verifying that goodness-of-fit values were close to expected theoretical values. The errors for the obtained parameters were estimated by Monte Carlo simulations with experimental noise levels. All numerical procedures were carried out in Matlab.

Results

Figure 1 shows experimental time courses for labeled glutamate obtained during 26 mM [1,6-$^{13}C_2$] glucose perfusion of melanoma cells cultured in a CST bioreactor.. Metabolic fluxes determined from kinetic curves were (in mmol/Lcell/hr): Ftca=10.3, Fpc=0.4, $F_{anaplerosis}$(at the level of Succinyl-CoA)=15.3, Fx(α-Ketoglutarate-Glutamate)=5.9, and Michaelis-Menten parameters for lactate transport kinetics- $V_{max}^{tr(lac)}$=204 and Km=3.5 mM. . TCA flux and especially anaplerotic exchange flux are high in melanoma cells. The estimated cellular metabolic rate of oxygen consumption (CMRO2) from two data sets was 34+/-2 mmol/Lcell/h compare to the experimental rate (measured polarographically from ΔpO_2) 33+/-4 mmol/Lcell/h. ATP production was calculated assuming a P/O ratio of 2.5 for NADH, 1.5 for $FADH_2$ and assuming equal fluxes through the malate-aspartate and glycerol-phosphate shuttles. Using the average values from the two experiments, we calculate rates of ATP production (CMR_{ATP}) as CMR_{ATP}(mitochondrial) = 174 mmol/Lcell/h; CMR_{atp}(glycolytic) = 150 mmol/Lcell/h, which corresponds to 54% of the energy coming from oxidative phosphorylation and 46% from aerobic glycolysis.

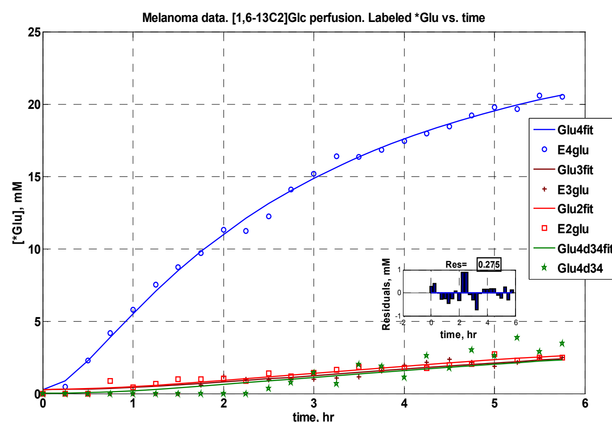

Fig. 1 [1,6-$^{13}C_2$]glc perfusion. Total glutamate C4,C3,C2 and doublet C4D34 labeling time courses. Continuous lines represent the best fits (4 curves) to the experimental ^{13}C MRS

Discussion and Conclusions

Under aerobic conditions glycolysis provides nearly as much ATP as oxidative phosphorylation; under *in vivo* conditions glycolysis may well prove to be the major source of tumor energy. This will vary from tumor line to tumor line, and the DB1 tumor is relatively oxidative compared to other melanoma lines. Since the cells were well oxygenated, whereas *in vivo* a substantial fraction of the cells will be hypoxic, we can anticipate increased contribution from the glycolytic pathway if these tumors exhibit a significant Pasteur effect. Our study suggests that Warburg assumption that tumors are deriving most of their energy from glycolysis is close to the truth. The model is validated by excellent agreement between model predicted and experimentally measured values of $CMRO_2$ and cytosolic glutamate pool size. The present ^{13}C metabolic model takes into account the additional information available from ^{13}C the multiplets in ^{13}C spectra. This leads to significantly increased precision in calculated metabolic fluxes. Also the new cumomer approach significantly reduces the number of kinetic isotopomer equations required to generate a full kinetic model in terms of participating fine structure ^{13}C multiplets and provides a more effective method for investigation of detailed mechanisms of cancer metabolism. Dynamic high-resolution MR spectra is very sensitive to changing/adding biochemical pathways and flux values, and cumomer modeling allows one to check precisely the feasibility of assumed general bionetworks and particular metabolic pathways.

References

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