In vitro monitoring of total choline levels in a bioartificial pancreas: \(^1\)H NMR spectroscopic studies of the effect of oxygen level on immunoprotected \(\beta\)TC3 insulinoma cells.

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Introduction

Readily available transformed cell lines show promise for the use in the construction of a bioartificial pancreas for the long-term treatment of diabetes mellitus, especially due to the fact that pancreatic islets from human and animal sources are in short supply. The \(\beta\)TC family of mouse insulinomas, specifically the \(\beta\)TC3 line, are an excellent cell choice for initial study of the design of these constructs, as well as the limitations of the effect of cell entrapment and environmental oxygen and nutrient supply over the long term. NMR allows the non-destructive monitoring of these intact constructs. When entrapped in an immunoprotected environment, such as alginate/poly-L-lysine/alginate (APA) beads, the cells can be implanted and are potentially useful in restoring normoglycemia. The new results presented are obtained from water suppressed \(^1\)H spectroscopy and provide the total choline and lactate levels under conditions of varying oxygen concentration in the perfusion media.

Methods

Cell culture \(\beta\)TC3 cells were obtained from the laboratory of Dr. Shimon Efrat at the Albert Einstein College of Medicine. Cells were cultivated in DMEM medium containing 20mM glucose, 15% heat inactivated horse serum, 2.5% fetal bovine serum, penicillin/streptomycin, 0.04mM choline chloride, and 6.0mM glutamine.

Entrapment \(\beta\)TC3 cells were entrapped in calcium alginate/poly-L-lysine/alginate beads with a diameter of 1.0mm ± 0.2mm.

Diphase extraction Cells from monolayers and cells in beads were extracted using the procedure by Tyagi et al. \(^2\)

Perfusion System The perfusion system consisted of a 22 ml packed bed bioreactor with supporting perfusion loop. Replenished medium is circulated at 37\(^\circ\)C through the bioreactor. This system maintains a constant level of nutrients, stable pH, and medium flow rate (30 ml/min). It also allows for repeated periods of hypoxia. Inlet and outlet oxygen levels of the bioreactor are monitored to obtain the oxygen consumption rate (OCR) of the \(\beta\)TC3 cells in APA beads. Lactate and glucose are monitored in the perfusion medium over the long-term culture (~30 days) for correlation with the NMR data.

NMR Spectroscopy NMR data was obtained for the perfused entrapped cell preparations from a SIS Co. 200/33 spectrometer. \(^1\)H water suppressed spectra were obtained by the use of an adiabatic spin echo sequence. This sequence employs solvent suppressive adiabatic pulses (SSAP) first demonstrated by Garwood et al. \(^3\) Information about metabolite levels along the bioreactor direction of flow was obtained by phase-encoding along this direction. \(^1\)H water suppressed extract spectra were obtained to confirm resonance assignments for the spectra of intact beads.

Results

Two separate cultures of entrapped \(\beta\)TC3 cells were used in these studies. A water suppressed adiabatic pulse sequence was employed to monitor total mobile choline species (total choline) and lactate levels as a function of distance along the axis of flow of our bioreactor containing APA beads of entrapped \(\beta\)TC3 cells over the ~30 day culture time under defined nutrient conditions. Hypoxic episodes for 4hrs and 24hrs were also carried out. During these episodes the ratio of total choline to isoleucine for the 4hr and 24hr episodes fell ~13% and 37% of the pre-hypoxic values, respectively. Lactate levels increased significantly. Upon return to normoxia, total choline levels returned to pre-hypoxic values. Figure 1 shows the ratio of (total choline/lactate) as a function of distance along the bioreactor length over the 30 day culture period. Initially, the level of total choline is constant from inlet to outlet, indicating a homogeneous distribution. As culture time increases, the outlet ratio drops relative to the inlet in a similar way to the outlet oxygen level. Due to the low choline concentration in the perfusion media, the total choline signal is primarily due to intracellular choline species. Lactate, however, is primarily extracellular. Due to the steady state concentration of lactate and constant perfusion flow rate, lactate level is constant along the bioreactor flow axis and the reduced ratio indicates reduced total choline.

Conclusions

Water suppressed \(^1\)H spectroscopy provides a novel view of \(\beta\)TC3 metabolism confined in APA beads under controlled perfusion conditions of known oxygenation. Total choline levels track the oxygen consumption rate of the culture over prolonged periods (~30 days). Both 4hr and 24hr hypoxic episodes show significant reduction in total choline with return to pre-hypoxic levels. Although OCR returns immediately, the return of total choline level requires a prolonged time period. The development of the total choline gradient across the bioreactor over time, coupled with significant reductions during 4hr and 24hr hypoxic episodes, suggest for the first time a significant correlation between total choline level and oxygen availability.

Figure 1. Total choline to lactate ratio for a 22ml bioreactor packed with entrapped \(\beta\)TC3 cells in APA beads as a function of distance from the bioreactor center.

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References