Probing mitochondrial disorders through 13C and 31P NMR analysis of extracts

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Synopsis:

Disorders of mitochondrial energetics have dire consequences for afflicted individuals. In this study we grew cell cultures from patients with mitochondrial disorders and labeled metabolic intermediates by incubating the cells in U-^{13}C-glucose. By performing NMR ^{31}P and ^{13}C spectroscopy on the cell extracts, information relevant to the cellular defect can be obtained. This study examines spectroscopic data generated from cell lines in the presence and absence of a compound to enhance carbon entry into the TCA cycle. It illustrates the potential for using NMR spectroscopic techniques to provide information relating to metabolic disorders.

Introduction:

The mitochondrion is the organelle responsible for vital cellular processes such as energy (ATP) production, fatty acid oxidation, and apoptosis. Mitochondrial disorders are the source of a vast array of degenerative diseases, and are a major challenge to the medical community because of the nature of the affliction and the difficulty in determining the etiology of the abnormality. Consequently, interventional therapies for patients with metabolic disorders are often tried empirically with limited success. Some compounds commonly used to enhance mitochondrial function are dichloroacetate (DCA), carnitine, dihydrolipoate, nicotinamide, CoQ_{10}, vitamins C, K_3, thiamine and riboflavin. Unfortunately, despite rigorous therapy, many patients never achieve adequate therapeutic gain.

This study was initiated to establish the utility of NMR spectroscopy of extracts taken from patients with mitochondrial mutations to probe the metabolic consequences of mitochondrial defects. NMR ^{31}P and ^{13}C spectroscopy is well suited to provide a quantitative assessment of cellular energy as well as glucose metabolism through intermediate labeling [1,2]. Here we present data on patients with mitochondrial disorders compared to a normal control cell line.

Materials and Methods:

Fibroblast cell cultures were grown and expanded *in vitro* from skin punch biopsies. The culture derived from a normal volunteer (*A*) was transformed with SV40, while the cultures from two patients with mitochondrial disorders (*B* and *C*) were of primary origin. B cells have a pyruvate dehydrogenase (PDH) deficiency, while the *C* cells have an unknown defect in oxidative phosphorylation. Confluent T-175 flasks of the fibroblasts were exposed for 4 hours to media containing 15 mM uniformly-labeled ^{13}C-glucose. To determine the impact of altering the cellular bioenergetics, some flasks of cells from each patient were exposed to ^{13}C-labeled media containing 5 mM DCA, a compound that augments the pyruvate flux through the mitochondrial PDH enzyme complex.

Extractions of the cells were performed as described by Tyagi *et al.* [3]. The aqueous portion of each extract was lyophilized, resuspended in a D_2O-H_2O solution, and placed in a 5 mm NMR tube. Spectroscopic ^{1}H, ^{31}P and ^{13}C NMR data of the extracts were acquired using a 5 mm broadband receiving coil in a 500 MHz vertical bore magnet equipped with a Bruker Avance console. Acquisition parameters were as follows: ^{1}H- sweep width = 6.67 kHz at 500.4 MHz, repetition time = 5 s, number of transients = 32; ^{31}P- sweep width = 8.09 kHz at 202.56 MHz, repetition time = 6 s, number of transients = 1024; ^{13}C- sweep width = 30 kHz at 125.84 MHz, repetition time = 6 s, number of transients = 7168. Waltz ^{1}H decoupling was applied throughout the ^{31}P and ^{13}C acquisitions.

Results and Discussion:

Table A summarizes results obtained from the ^{31}P data. Compared to the control cells *A*, *B* cells exhibited greatly reduced signals from ATP, phosphomonoesters and phosphodiesters. As *B* cells are known to have a PDH defect, treatment of DCA was expected to enhance the flux of pyruvate to the tricarboxylic acid (TCA) cycle. This therapy did increase the formation of glutamate species in *B* cells (indicating an increased entry of pyruvate into the TCA cycle), but this did not significantly alter ATP levels or ATP/ADP. There was a significant increase in the P_i of the DCA-treated *B* cell line compared to the untreated *B* cells. This is consistent with increased hydrolysis of ATP, suggesting that any amplified TCA activity was insufficient to overcome the energetic need of the cells. The *C* line exhibited low absolute ATP levels, with an ATP/ADP of ~4.2. Additionally, the ^{31}P spectra showed elevated P_i and reduced phosphomono- and –diesters, though none were as low as those of *B* cells. The glutamate levels of the *C* cells were also low. DCA therapy had no observable effect on this cell line, consistent with the fact that this line had a defect in the oxidative phosphorylation pathway, and not in PDH activity. Initial "TCA-calc" modeling analysis of the glutamate ^{13}C labeling patterns of the cell lines [**Fig. 1**] indicates that the *C* cells have the lowest fractional flux of pyruvate through PDH (0.49, compared to 0.58 in the *A* and *B* lines), though further modeling is warranted. The DCA molecule was observed by ^{1}H and ^{13}C in spectra of treated cells.

In summary, this preliminary study illustrates the potential of NMR spectroscopic techniques to elucidate biochemical consequences of inborn metabolic disorders. Additional patients with a range of mitochondrial defects will be assessed in similar fashion to create a database relating spectroscopic information and metabolic errors. In the future, information from studies such as this may allow for the tailoring of a rational efficacious therapy for individuals afflicted with defined mitochondrial defects.

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References:

[1] Malloy, C.R. et al. J. Biol. Chem. **263**: 6964, 1988

[2] Szwergold, B.S. Annu. Rev. Physiol. **54**: 775, 1992

[3] Tyagi, R.K., et al. Magn. Reson. Med. **35**: 194, 1996

Fibroblast Cell Lines	ATP/ADP	P_i
A (control line)	~5.5	98
A + DCA	~5.5	101
B (PDH defect)	~1.4	361
B + DCA	~1.4	689
C (oxidative phosphorylation defect)	~4.2	190
C + DCA	~4.5	200

Table A: Effect of 5 mM DCA therapy on fibroblast cultures. P_i is referenced to the β-NTP of each culture tested (defined as 100).

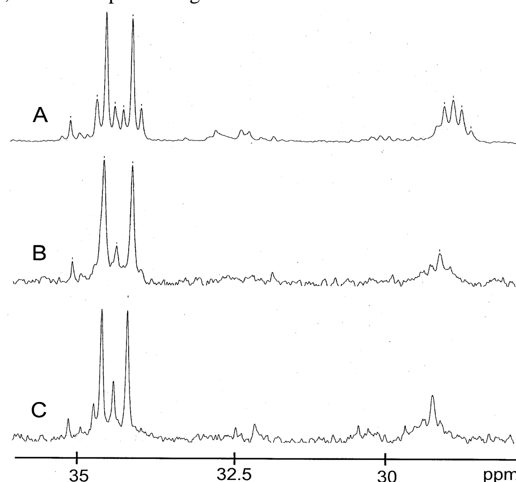

Figure 1: ^{13}C spectra from extracts of the cell lines representing the regions where the C3 (~28 ppm) and C4 (~34 ppm) of glutamate resonate.