INTRODUCTION

• Motor Neurone Diseases (MND) is a fatal neurodegenerative disorder characterized by loss of motor neuron groups within the spinal cord and brain

• The majority of MND cases are sporadic but around 10% are familial in origin linked to mutations in a number of genes

• The best described is mutation of Cu, Zn superoxide dismutase (SOD1), responsible for approximately 20% of all familial cases

• Oxidative stress and metabolic dysfunction have been implicated in the pathology of the disease

• Peripheral tissues have been shown to recapitulate disease pathology in a variety of neurodegenerative diseases

Aims

• To create a bioenergetic profile of MND patient SOD1 fibroblasts

ASSAYS

• Cell model-SOD1 113T fibroblasts isolated from MND patients

• Cellular oxidative stress assays performed using DCF fluorescence

• Mitochondrial and metabolic functional assays performed using a Seahorse Bioanalyser, measuring oxygen consumption rate (OCR) and extracellular acidification rate (ECAR)

• Addition of the ATP synthase inhibitor oligomycin, the mitochondrial uncoupler FCCP and the respiratory chain inhibitor rotenone, allowed the contribution of oxygen consumption to ATP synthesis and the respiratory capacity of the mitochondria to be assessed

• Total ATP and mitochondrial specific ATP levels were also measured

Results

• The SOD1 mutation confers greater susceptibility to oxidative stress than age and sex matched controls (Figure 1).

Conclusions

• MND SOD1 patient fibroblasts show higher susceptibility to oxidative stress

• SOD1 fibroblasts have reduced mitochondrial respiration and spare respiratory capacity.

• Levels of ATP produced by oxidative phosphorylation are reduced in SOD1 fibroblasts

• Switching to galactose increased mitochondrial respiration and spare respiratory capacity in SOD1 fibroblasts but also increased uncoupling

• Long chain fatty acid oxidation is reduced in SOD1 fibroblasts

• Starvation reduces FAO in control cells

• SOD1 patient fibroblasts recapitulate the metabolic defects observed in the CNS and muscle

SOD1 mutation in MND patient fibroblasts shifts energy generation from oxidative phosphorylation to glycolysis

Scott R Allen, Sandeep Rajan, Lynn Duffy, Heather Mortiboys, Andrew Higginbottom, Andrew J.Grierson and Pamela J Shaw

Department of Neuroscience, University of Sheffield

Figure 1. Basal cellular stress in SOD1 fibroblasts. Oxidative stress under basal (+) and glutamine withdrawn (-) conditions. Oxidative stress measured by addition of DCF to the cells. Data analyse by two way ANOVA with bonferroni post-tests, *P<0.05.

Figure 2. Metabolic analysis using an XF24 Seahorse Bioanalyser. A. Assay mechanics. B. Bioenergetic profile (BEP) design for OCR and ECAR

• Mitochondrial and metabolic analysis was performed on a Seahorse Bioanalyser which measures oxygen consumption (OCR) and extracellular acidification (ECAR-a measure of glycolytic flux) simultaneously. Mitochondrial and metabolic function was assessed under glucose and galactose conditions growth conditions. The contribution of long chain fatty acid oxidation to mitochondrial oxygen consumption was measured under physiological and starved conditions. The effect of hypoxia on mitochondrial function was also assessed.

Figure 3. Effect of SOD1 mutation on metabolic function with glucose. A. Mitochondrial respiration. B. Coupled respiration. C. Spare respiratory capacity. D. Glycolytic ECAR. Data presented as mean with std deviation n=6. *P<0.05 unpaired t test analysis.

Figure 4. ATP levels in SOD1 fibroblasts. A. Total ATP levels were measured using an ATPLite kit from PerkinElmer. B. ATP levels from oxidative phosphorylation. 1mM iodoacetate (IAA) was incubated with the cells for one hour. Data presented as mean with std deviation. n=5. Statistical analysis by unpaired t test and one way ANOVA with bonferroni post test analysis.

Figure 5. Contribution of fatty acid oxidation to cellular respiration. A. Malonyl CoA inhibition of OCR. B. Effect of glucose starvation on OCR. Data presented as mean with std deviation n=3. ***P=0.001, **P=0.01. *P=0.05 one way ANOVA with bonferroni post test analysis.