Final report

Title of project

Investigating mitochondrial function using tissue respirometry in critically ill patients

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Background

A growing body of experimental evidence suggests that mitochondrial function is modified early in critical illness and that dysfunction is related to clinical outcomes (1) (2). Defining and measuring mitochondrial function in a clinical context is challenging. Traditionally, studies assessing the relationship between mitochondrial function and clinical outcomes in critically ill patients have been limited to snapshot quantification of the content or activity of mitochondrial components in frozen tissues but static markers do not reflect the integrated and dynamic function of these complex intracellular organelles, which is influenced by their structural relationships and biochemical environment within the cell. Respirometry offers an alternative approach to exploring integrated mitochondrial function in a dynamic way, through measurements of oxygen consumption rates in fresh tissue or cells (3). The aim of this project was to establish whether this technique is feasible in the context of the intensive care unit. A systematic review was initially carried out to form the basis of a pilot observational study on the intensive care unit at the Royal Free Hospital.

Methods

A search of the MEDLINE and PubMed databases (1960 to August 2016) and a manual review of reference lists were conducted to find experimental studies using respirometry in samples extracted from critically ill patients. Full text articles were included in the review if the title and/or abstract reported the use of the respirometry technique to assess mitochondrial function, the method of respirometry was clearly defined in the methods section and the cells were extracted from critically ill patients requiring treatment in an intensive care unit.

Results

This search thus yielded six relevant studies. Cohorts included patients in both early (within 48 hours of admission to the intensive care unit) and late phases of critical illness. Cell types analysed included platelets, peripheral blood mononuclear cells and skeletal muscle. Experimental protocols used intact and permeabilised cells, and various sequences of substrate, inhibitor and uncoupler titrations (SUIT) to generate different respiratory states. Respirometry indices measured in critically ill patient cohorts were compared to controls, and between different timepoints during critical illness (within 48 hours, days 2-3, days 6-7 and beyond 2 weeks). Several studies investigated the relationship between mitochondrial phenotype and clinical outcomes. Respirometry indices were also expressed relative to putative markers of mitochondrial content (such as mitochondrial DNA, citrate synthase activity and cytochrome c). Studies were small in size (subject numbers ranged from 8 to 28), and there was significant heterogeneity in the study design, cell type, isolation procedures, experimental conditions (temperature, respiratory media and oxygen concentration), experimental protocols and specific indices of mitochondrial function generated (as well as

the nomenclature used to describe them), and quality control procedures. The variation in study design made it difficult to compare results across studies.

Conclusion

Respirometry is a method of characterising integrated and dynamic function of mitochondria within their intracellular context, and has been used to investigate changes in mitochondrial function during critical illness in human patients. Use of the technique in this context would benefit from standardised protocols and terminology to aid comparison between studies. The findings of this systematic review formed the basis of a pilot observational study investigating changes in mitochondrial function during critical illness (Tissue metabolism and blood flow in critical disease, TIMELORD, sponsored by the Intensive Care Society and Royal Free Charities). This review, and the preliminary results from the study itself, were presented at the Mitochondrial Physiology Society Training School, Obergurgl, Austria 2017, with assistance from the NIAA Belfast Fund. This training school and workshop forms part of an international collaborative between basic scientists and clinicians using respirometry in different cell and animal models of disease, as well as in the clinical setting. Further investigation is ongoing, and this work is part of an MD project with University College London (fellowship sponsored by the London Clinic) and when data collection is completed, a manuscript will be submitted for publication.

1. Brealey D, Brand M, Hargreaves I et al. Association between mitochondrial dysfunction and severity and outcome of septic shock. Lancet. 2002;360:219-223.
2. Carre JE, Orban JC, Re L et al. Survival in critical illness is associated with early activation of mitochondrial biogenesis. Am J Respir Crit Care Med. 2010;182:745-751.
3. Pesta D, Gnaiger E. High-resolution respirometry: OXPHOS protocols for human cells and permeabilized fibers from small biopsies of human muscle. Methods Mol Biol. 2012;810:25-58.