A295 - Reduced Cellular Respiration and ATP Production in an In Vitro Model of Sepsis

V Herwanto 1; Y Wang 1; M Shojaei 1; B Tang 2; A McLean 2

1University of Sydney, Westmead Institute for Medical Research, Westmead, Australia, 2Nepean Hospital University of Sydney, Nepean Clinical School, Department of Intensive Care, Kingswood, Australia

Introduction:
Leukocyte dysfunction may play a role in sepsis pathogenesis. Established evidence showed that leukocyte dysfunction leads to reduced immune response and consequently an increased sepsis-related mortality. Impaired metabolism has been recently proposed as one possible mechanisms underpinning leukocyte dysfunction in sepsis. In this study, we investigated the global changes in leukocyte metabolism in sepsis, using an established in vitro model of lipopolysaccharide (LPS) stimulation.

Methods:
Peripheral blood mononuclear cells (PBMC) were isolated from healthy volunteers (n=4) and incubated with 62.5 ng/mL LPS. Mitochondrial respiration was measured using Agilent Seahorse XF Analyzer (Cell Mito Stress Test Kit). Total cellular oxidative stress was measured using DCFDA Cellular Reactive Oxygen Species (ROS) Detection Assay Kit (Abcam) and mitochondrial superoxide was measured using MitoSOXTM (Life Technology). Apoptosis was measured by Annexin V-FITC Apoptosis Detection Kit (Abcam). Evaluation of oxidative stress and apoptosis were performed using BD FACSCanto flow cytometer and flow cytometry data was analyzed using FlowJo Software V10.

Results:
LPS stimulation of PBMC from healthy volunteers showed a trend of decrease in both oxidative phosphorylation and cellular respiration (Figure 1). This decrease in cellular metabolism was accompanied by a trend towards an increase in cell death in the stimulated leukocytes (Figure 2). The increase in cell death was associated with an increase in oxidative stress (total and mitochondria) (Figure 2), suggesting that the adverse effect of LPS on cellular metabolism may be mediated by an imbalance in redox potential.

Conclusion:
The LPS stimulation model could provide a useful approach to study the effect of sepsis on leukocyte metabolism. Further study is required to better understand the mechanism of reduced leukocyte metabolism, including the possible role of oxidative stress in reducing cellular respiration and causing leukocyte cell death.

Image 1:

Cellular metabolism as measured in oxygen consumption rate (OCR) (n = 4). Basal denotes energetic demand of the cell under baseline condition; spare respiratory capacity denotes the capability of the cell to respond to energetic demand; proton leak denotes remaining basal respiration not coupled to ATP production, can be a sign of mitochondrial damage; ATP production shows ATP produced by the mitochondria to meet the energetic need of the cell.

Image 2:
Number of apoptotic cells, total cellular ROS, and mitochondrial superoxide as measured by Annexin V, DCFDA, and MitoSOX (n = 4).