Endotoxin-induced alterations in renal oxygen consumption: an ESR oximetry study

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1. BACKGROUND

The kidney is one of the most injured organs in critically ill patients. For those patients, sepsis and septic shock are the most important cause of acute renal injury (ARI), and accounts for more than 50% of cases of ARI in intensive care units. ARI is associated with a high mortality. Indeed, the combination of ARI and sepsis is associated with a 70% mortality, as compared with a 45% mortality among patients with ARI alone [1].

During a septic shock, the kidney is faced with unique challenges in the maintenance of homeostasis of intrarenal oxygenation. Recent research activities in the pathophysiological mechanism of ARI emphasize the central role of hemodynamic and inflammatory events in septic shock. More particularly, two mechanisms have been postulated to explain the inability of the injured kidney to extract oxygen: tissue hypoxia and cellular energetic metabolism dysfunction [2].

The unbalanced homeostasis between nitric oxide (NO), reactive oxygen species (ROS) and renal oxygenation, could form a major component of the development of ARI [3]. However, it is the O2 content that is of crucial interest as it is a substrate for the cell energy adenosine triphosphate (ATP) production.

2. IN VITRO MODEL

The present investigation was carried out to characterize renal oxygen consumption in a model of septic ARI by setting up an in vitro model.

HK-2 cells, derived from human proximal tubular cell (PTC) of a human kidney, were cultured in DMEM supplemented with 10% FBS, 2 mM L-Glutamine, 100 U/ml Penicillin and 100 µg/ml Streptomycin.

To stimulate the pro-inflammatory rate of PTC, cells were incubated with lipopolysaccharide (LPS) from E. coli O55:B5. LPS is released from the gram-negative bacteria and is one of the major initiators inflammatory response during sepsis. LPS is known to produce an early rise in cytokines through activation of Toll-like receptor [4]. One concentration (1 µg/ml) and different incubation time (1h, 6h and 18h) were tested.

3. ESR OXIMETRY

Electron spin resonance (ESR) oximetry is a very sensitive method that permits continuous monitoring of cellular oxygen consumption [5].

The method is based on the variation of the line width of a paramagnetic material with the oxygen concentration in a closed system. An increase in oxygen concentration increases the ESR spectra line width of the probe.

In this study, a neutral nitroxide, 1H-N-PDT, was used as the oxygen sensor. Changes in its ESR spectrum was calibrated with partial pressure of oxygen (PO2) and than used to measure the oxygen consumption of the HK-2 cells. All spectra were recorded on a Bruker EMX ESR spectrometer.

4. RESULTS

These results show that endotoxin has a direct effect on the rate of oxygen utilization by HK-2 cells.

As it can be seen, incubation with LPS reduced the oxygen consumption rate in HK-2 cells by approximately 30% in a time-independent manner.

This decrease induced by LPS could be due to a decrease in the number of viable cells. However, routinely the cell viability was 85-95%.

A possible interpretation is that this change may be in relation with a metabolic down-regulation. Renal energetic are deranged in sepsis not just because O2 delivery is impaired but perhaps also because the ability of cells to utilize O2 is compromised [6].

5. CONCLUSION

To develop a model of septic ARI, the effect on oxygen consumption of LPS-treated renal cells has been tested. In this study, we have used the technique of ESR spectroscopy coupled with oxygen-sensitive probes for the evaluation of oxygen uptake and utilization in HK-2 cells treated with endotoxin. We have shown that oxygen consumption rate decrease quite markedly in LPS-treated cells. This decrease may be in relation with a metabolic down-regulation. An acquired defect in oxidative phosphorylation caused by cytochrome oxidative inhibition could prevent cells from using oxygen for adenosine triphosphate production and potentially cause sepsis ARI [6].