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

Caroline Quoilin[1], Angeliki Grammenos[1], Bernard Gallez[2], Maryse Hoebeke[1]

[1] Laboratory of Biomedical Spectroscopy, Department of Physics, University of Liège, Liège, Belgium
[2] Laboratory of Biomedical Magnetic Resonance, Louvain Drug Research Institute, University of Louvain, Brussels, Belgium

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 O_2 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 sate of PTC, cells were incubated with lipopolysaccharide (LPS) from *E. coli 055: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.

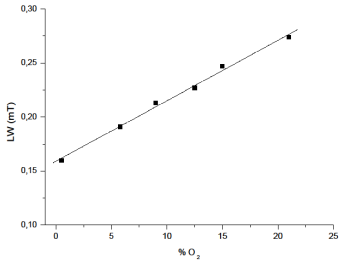
3. ESR OXIMETRY

Electron spin resonance (ESR) oximetry is a very sensitive method that permits continuous monitoring of cell 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, ^{15}N-PDT, was used as the oxygen sensor. Changes in its ESR spectrum was calibrated with partial pressure of oxygen (pO_2) and than used to measure the oxygen consumption of the HK-2 cells. All spectra were recorded on a Brucker EMX ESR spectrometer.

Figure 1 : The ^{15}N-PDT probe was calibrated at various partial pressure of oxygen (between 100% nitrogen and air) so that the line width (LW) measurements can be equated with oxygen concentration (pO_2) at any value. *This calibration has been done by the Laboratory of Biomedical Magnetic Resonance* [5].



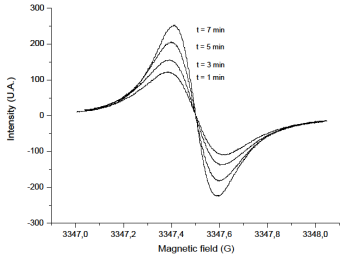


Figure 2 : When ^{15}N-PDT is added to the cell suspension, the nitroxide is distributed throughout the extracellular and intracellular space, and the resulting line width reports on $[O_2]$. Its line width decreases over time traducing the HK-2 oxygen consumption.

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 O_2 delivery is impaired but perhaps also because the ability of cells to utilize O_2 is compromised [6].

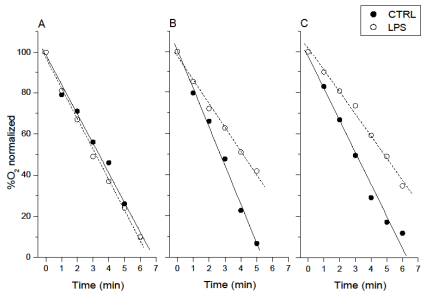
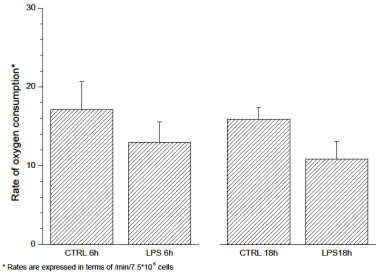


Figure 3 : Oxygen consumption rate of HK-2 cells measured by ESR oximetry is given by the mean slope of the graph that presents the variations in the percentage of oxygen over time. 7.5 x 10^6 cells were incubated in absence (●) or in presence (○) of 1 µg/ml LPS during 1h (A), 6h (B) and 18h (C). After 6h of incubation with the endotoxin, the rate of oxygen consumption is reduced in a significantly manner.

Figure 4 : LPS-treated cells consume oxygen significantly more slowly than control cells. Oxygen consumption decrease quite markedly in treated cells: from 17.12 ± 3.54/min/7.5 x 10^6 cells in the control group to 12.94 ± 2.62 /min/7.5 x 10^6 cells in the 6h incubation time group and from 15.93 ± 1.49 /min/7.5 x 10^6 cells in the control group to 10.86 ± 2.2/min/7.5 x 10^6 cells in the 18h incubation time group. This decrease is incubation time independent, as far as the



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 tri-phosphate production and potentially cause sepsis ARI [6].

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