ESR SPECTROSCOPY FOR THE STUDY OF AN INFLAMMATION-INDUCED AKI CELLULAR MODEL

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1. Background

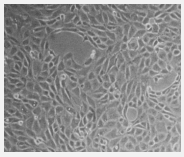
Sepsis can be considered as a heterogeneous disease process generated by a complex interaction of pathogen and host inflammatory response. It may lead to organ dysfunction distant from the primary site of infection and cause downstream effects such as multiple organ failure [1].

The kidney is one of the target organs of sepsis which is well-known to be a risk factor for the development of acute kidney injury (AKI). Recent research activities in the mechanisms involved in the development of AKI in sepsis emphasize the central role of hemodynamic and inflammatory events.

More particularly, two mechanisms are suggested to explain the inability of the injured kidney to extract oxygen: tissue hypoxia and cellular energetic metabolism dysfunction [2]. Our working hypothesis of the pathophysiology of AKI is based on cellular respiratory dysfunction due to the inflammatory response inherent to sepsis.

2. In Vitro Model

We developed an *in vitro* model of inflammation-induced acute kidney injury using HK-2 cells exposed to lipopolysaccharide (LPS) [3].



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 exposed to 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.

One concentration (1 µg/ml) and different incubation time (1h to 24h) were tested.

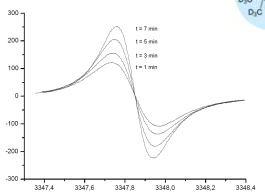
3. ESR Oximetry

Objective

To characterize renal oxygen respiration in this inflammation-induced model of AKI

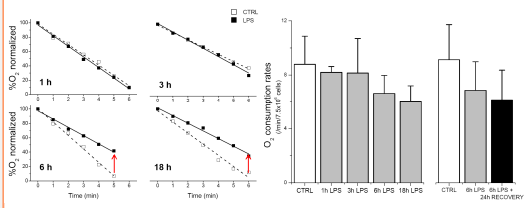
Principle

- O_2: paramagnetic molecule with 2 unpaired electrons and very rapid relaxation time
- ^{15}N-PDT: oxygen sensor



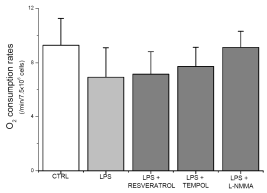
- O_2 influences spin-lattice and spin-spin relaxation times of ^{15}N-PDT
- Heisenberg: An increase in oxygen concentration increases the ESR spectra line width of the probe

Influence of LPS [3]



- The basal respiration of LPS-treated HK-2 cells is altered and presents a strong decrease in the oxygen consumption rates
- This cellular respiration alteration persists even after the stress factor was removed
- This irreversible decrease in renal oxygen consumption after LPS challenge may be related to a pathologic metabolic down-regulation such as a lack of oxygen utilization by cells [4]

Influence of Drugs



- Resveratrol and Tempol: Antioxidants
- L-NMMA: Nitric oxide synthase inhibitor

- When treated with resveratrol and tempol, the oxygen consumption rate measured remains low
- When treated with L-NMMA, HK-2 cells recover a normal cellular respiration
- The signaling pathway by which LPS affects HK-2 cells metabolism mainly involves NO rather than ROS

4. Spin Trapping

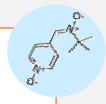
Objective

To detect inflammatory mediators (ROS and NO) thought to play a role in this inflammation-induced model of AKI

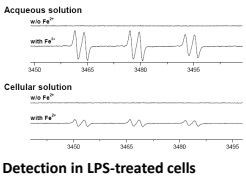
Principle

	Spin trap	Free radicals →	Spin adduct
Magn. Prop.	Diamagnetic	Paramagnetic	Paramagnetic
Life time	∞	Very short	Long (mn, h)

ROS Detection with POBN/EtOH

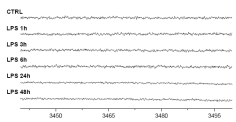


Test Reaction: $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\cdot}$



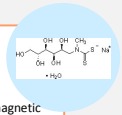
- POBN localizes inside cells and is very specific to OH·
- EtOH scavenge OH· and the ethoxy radical is trapped by POBN to form POBN-ethoxy adducts (a_N=15.7 G; a_H=2.7G)

ROS Detection in LPS-treated cells

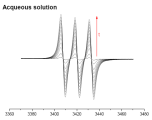


- After LPS treatment, no spectrum of POBN-ethoxy adducts was observed in HK-2 cells
- ROS may not be the major mediator in the induction of LPS stress

NO Detection with MGD/Fe^{2+}



Test Reaction: $SIN-1 \rightarrow SIN-1c + NO^{\cdot}$



- Although NO· is a paramagnetic compound, it is ESR silent at T_{amb} in aqueous solution
- When NO· is trapped with $\{Fe^{2+}\text{-}(MGD)_3\}$, the resulting nitrosyl complex is stable and ESR-detectable

NO Detection in LPS-treated cells

- Using the Griess test, we have observed that the decrease in O_2 consumption is accompanied by an increased nitric oxide (NO) production
- This observation has to be confirmed by ESR spectroscopy

Conclusions

Overall, ESR spectroscopy and the model of HK-2 cells exposed to LPS display some key features of inflammation-induced acute kidney injury:

1. Alteration in the renal cellular respiratory function
2. Role of NO· rather than ROS in the signalling pathway
3. Role of INOS in the generation of NO·

Open Questions

- Since L-NMMA permitted the HK-2 cells to recover a normal cellular respiration, does it blocked NO generation?
- Is it possible to detect the activation of inducible NO synthase?
- Are mitochondrial alterations the mechanism of HK-2 cells basal respiration perturbations during inflammation?

Acknowledgements

The authors thank Laboratory of Biomedical Magnetic Resonance for the use of the Brucker EMX ESR spectrometer.

This wok was supported by Grants from the Belgian National Fund for scientific Research (FNRS) and the University of Liège (ULg).

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