In vitro ESR measurements: powerful tools to study toxic effects on cells

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Introduction

The ESP Bruker 300 used for the different experiments.

***Electron Spin Resonance (ESR)** spectroscopy is a technique for studying chemical species that have one or more unpaired electrons.*

*The detection of such species can be direct or indirect (**spin trapping**).*
*The incorporation of probes containing an unpaired electron is also used to measure the influence of the environment on these probes (**spin labeling** or **oximetry**).*

The ESR technique has been developed to investigate the biomedical and pharmacological field:

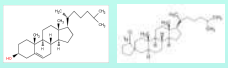
*- ESR allows the quantification and qualification of **reactive oxygen species (ROS)** produced by cells themselves, by **oxidative stress (drug)** or by **therapeutic treatments** (photodynamic therapy(1)).*
*- ESR can carry out measurements of **membrane fluidity** of cells or cellular models such as liposomes or micelles. This method can allow us to see **the change of viscosity** induced by **drug way**, **diets** or **pathology**.*
*- ESR oximetry permits continuous monitoring of **cell oxygen consumption**. This very sensitive method can detect changes in the oxygen consumption rate when cells are subjected to **toxic stress**.*

Application 1: SPIN LABELING

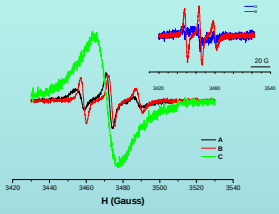
Effect of a Cyclodextrin

Structure: Cyclodextrins are amphiphilic molecules. They possess an hydrophobic cavity that permits to encapsulate other hydrophobic molecules.

In the present study we investigate the interaction between RAMEB (per (2, 6- O - diméthyl) random- b- cyclodextrin) **and HCT-116 cells.**



Molecule of cholesterol *Probe of cholestane*



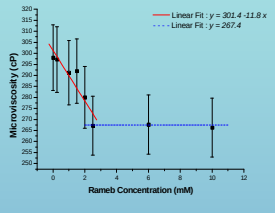
The only one large distorted peak observed results of an important spin-spin nitroxide interaction favored by a high local concentration of cholestane in the HCT-116 membrane (C). This result confirms that cholesterol is organized in lipid raft in the cell membrane.

The evolution of probe ESR spectrum in cells incubated with Rameb (A) demonstrates that cholestane is extracted from the lipid rafts and formed aggregates with the Rameb (A & B).

Spectrum of cholestane solubilised in PBS and containing Rameb (B).
Spectrum of cholestane solubilised in PBS (D).

The cholesterol extraction due to the Rameb incubation of cells leads to a decrease in their membrane microviscosity.

Microviscosity is directly linked to the cholesterol content in the membrane(2).



Application 2: SPIN TRAPPING

Effect of Preconditioning

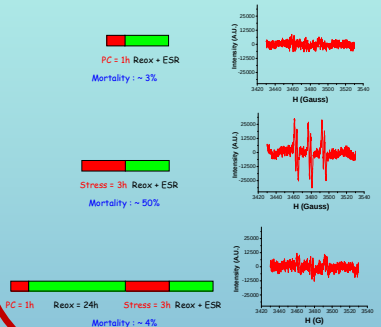
Definition: A short period of sublethal stress can protect an organ against a more prolonged period of stress (eventually lethal).
This sublethal stress is called **Preconditioning (PC)**.

Clinical targets of PC: Heart (infarct), Brain (stroke,thrombosis), Liver, Intestine, ...

Nature of stresses: Ischemia (O_2, glucose), Hypoxia (O_2), Hypoglycemia (glucose), Anesthesia,...

In the present study we performed anoxia (1h or 3h) on Neuro-2a cell line. The detection of ROS was achieved by a combination of ethanol (2%) and POBN spin trap one hour after anoxia (reoxygenation period).

Anoxia-reoxygenation cycles **ESR spectra**



The benefit offers by PC (1h anoxia) before a strong stress (3h anoxia) is obvious on ESR spectra.
A small period of anoxia reduced dramatically the production of ROS.

This reduction is immediately observed by a better cellular viability.

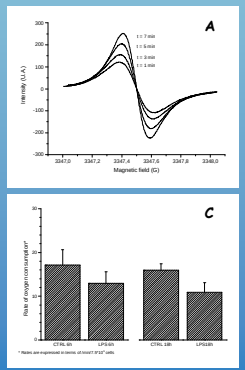
Application 3: OXIMETRY

Effect of an Endotoxin

Clinical research: The kidney is one of the most injured organs in patients with septic shock. This clinical complication is called septic acute renal injury (ARI).

Hypothesis: The injured kidney is unable to extract oxygen due to tissue hypoxia or/and cellular energetic metabolism dysfunction(3).

The present investigation was carried out to characterize renal oxygen consumption in a septic ARI by setting up an *in vitro* model. Human proximal tubular cells (HK-2) were incubated with lipopolysaccharide (LPS) during 6h or 18h.



The line width of the probe (^{15}N-PDT) varies with the oxygen concentration: when [O_2] increases, its line width increases (**A**).

The probe is used to measure the oxygen consumption of the HK-2 cells (**B**).

LPS-treated cells consume oxygen significantly more slowly than control cells (**C**). This observation suggests a down-regulation of the cells metabolism.

(1) Guelluy, P. H., M. P. Fontaine-Aupart, A. Grammenos, S. Lecart, J. Piette, and M. Hoebeke. 2010. Photochem Photobiol Sci 9:1252-1260.
(2) Grammenos, A., A. Mouithys-Mickalad, P. H. Guelluy, M. Lismont, G. Piel, and M. Hoebeke. 2010. Biochem Biophys Res Commun 398:350-354.
(3) Rudiger, A., M. Stotz and M. Singer, 2006. Swiss Medical Weekly 138: 629-634.

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