Funded by the European Union
NextGenerationEU

SUMMIT
SOFIA UNIVERSITY
MARKING MOMENTUM
FOR INNOVATION AND
TECHNOLOGICAL TRANSFER

SOFIA UNIVERSITY -
MARKING MOMENTUM
FOR INNOVATION AND TECHNOLOGICAL TRANSFER

National Recovery and Resilience Plan
OF THE REPUBLIC OF BULGARIA

Verification of a new redox-sensitive contrast probe for visualization and evaluation of redox state and oxidative stress in biological objects through magnetic resonance techniques

Head of the research group
Assoc. Prof. Dessislava Lazarova
Members of the group
Prof. Rumiana Bakalova
Researcher R1 Dimitar Ivanov
Assist. Prof. Daiana Yankova-Pushkarova

INTRODUCTION

The concept of "Redoxome" dates since 2005-2010. It is directed to defining the pathways and networks that organize the functioning of cells, tissues, and organisms. The main goal is to obtain quantitative information on redox-regulated proteins (proteins with highly reactive cysteine residues whose thiol oxidation state controls the function of proteins and their biochemical networks), as well as on unstable, quasi-stable, and redox-active species (e.g. reactive oxygen and nitrogen species, ROS/RNS), that determine the redox environment of cells and tissues.

Currently, "redox theranostics" is one of the fastest growing areas of biomedicine. Its main goal is to visualize, analyze and modulate the redox-state of cells, tissues and body fluids – a crucial factor for slowing aging and fighting various pathologies, such as: malignat, neurodegenerative, autoimmune, atherogenic, infectious, etc., characterized by redox imbalance and induction of oxidative stress. This makes the redox-state a valuable diagnostic marker and therapeutic target.

Numerous synthetic or genetically encoded contrast probes have been described to form detectable products reflecting the localization and level of a particular redox-active substance or group of substances in the studied biological target. Detection of most of these probes (e.g., fluorescent and chemiluminescent) is feasible with high sensitivity and resolution, but mostly in vitro and with many difficulties and limitations in vivo. With another group of contrasts (e.g., nuclear and ultrasound), in vivo detection is possible with high sensitivity but low spatial resolution (e.g., nuclear) or high spatial resolution but low sensitivity (e.g., ultrasound), with results varying depending on the operator.

Cyclic nitroxides are one of the most attractive redox-sensitive probes. They are sterically hindered and resonance stable and can be registered in vitro and in vivo by magnetic resonance techniques such as electron paramagnetic resonance (EPR) and magnetic resonance imaging (MRI). Currently, nitroxide-enhanced magnetic resonance imaging is one of the most sensitive and specific methods for analyzing the redox-state in cells, tissues and body fluids, as well as for assessing redox imbalance and oxidative stress.

PROJECT GUIDELINES

Our project aimed at verifying a new redox-sensitive contrast probe (redox sensor, RS) for visualization and quantitative analysis of redox-state and oxidative stress in biological objects in vitro and in vivo using nitroxide-enhanced EPR and MRI. RS was developed by the project team in collaboration with Japanese scientists. The sensor is based on the use of natural cyclodextrin as a matrix, onto which stable paramagnetic cyclic nitroxide residues (TEMPO) and a triphenylphosphonium residue are conjugated, ensuring penetration of the probe into cells and tissues. Since cyclodextrins are generally recognized as safe in the EU, Japan and FDA for applications in pharmaceuticals and dietary supplements, we believe that they are a suitable matrix for the development of a new generation of low-toxic and even non-toxic multispin contrast probes with high potential for application in clinical practice. Some cyclic nitroxides (TEMPO derivatives) are currently registered in over 10 clinical trials approved by the FDA, which also confirms their safety within certain concentration range.

The project is of significant clinical and social importance due to the following circumstances:

- Oxidative stress and the redox-state of cells, tissues, and biological fluids are some of the most extensively analyzed parameters in biomedicine and fall into one of the fastest growing sectors of research and development in the biotechnological industry.
- Oxidative stress and redox homeostasis are key factors in the pathogenesis of many pathologies. Monitoring and controlling these two parameters can be crucial for the effectiveness of therapy, eliminating harmful side effects and improving the quality of life of patients.
- The project opens new horizons for a wider application of EPR imaging and MRI in biomedical diagnostics, e.g. for "redox imaging". Therefore we expect the promotion of the results to provoke the interest of companies developing these technologies.

METHODOLOGY

RS is characterized by redox cycle, which determines the dynamics of the EPR/MRI signal and makes the probe suitable for redox imaging in biological objects. The nitroxide redox cycle links the conversion between two forms in vivo:

(a) paramagnetic (radical), characterized by EPR/MRI contrast;
(b) diamagnetic (hydroxylamine) without EPR/MRI contrast.

Various endogenous reducers can directly or indirectly convert the nitroxide radical into hydroxylamine, which is accompanied by EPR/MRI signal decay. In contrast, the interaction of hydroxylamine with oxidizers (mainly superoxide) restores the radical form of nitroxide and its EPR/MRI signal. The rapid decay or complete disappearance of the EPR/MRI signal in biological specimens is a marker of their high reducing capacity. The slow decay of the signal or the lack of such an effect is a marker of a high oxidative capacity in the studied target.

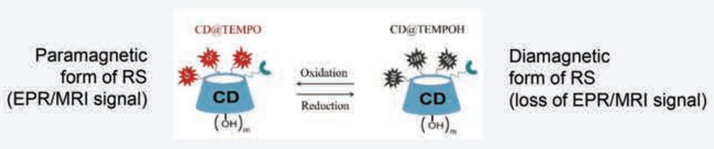


Figure 1. Redox cycle of our nitroxide-based redox sensor (CD@TEMPO) and dynamics of its EPR/MRI signal.

Experiments were performed on healthy mice and mouse model of acute inflammation (LPS-treated mice):

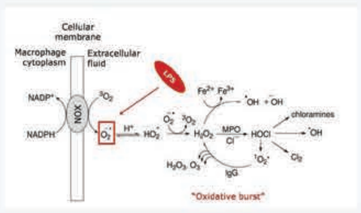
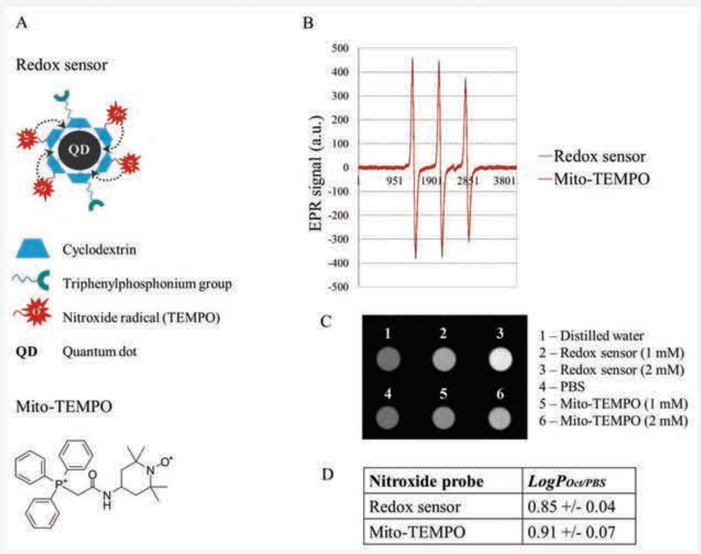


Figure 2. "LPS mouse model of acute inflammation", accompanied by induction of "oxidative burst" in LPS–activated macrophages: molecular mechanism. LPS - lipopolysacharide

RESULTS



Nitroxide probe	$LogP_{Oct/PBS}$
Redox sensor	0.85 +/- 0.04
Mito-TEMPO	0.91 +/- 0.07

Figure 3. Characteristics of redox sensor (RS) and mito-TEMPO (reference contrast probe): (A) Structure of RS and chemical formula of mito-TEMPO; (B) Electron paramagnetic resonance (EPR) spectra of RS and mito-TEMPO, registered in PBS in equal concentrations (0.1 mM); (C) T_1W MRI on phantoms, containing: RS or mito-TEMPO in equal concentrations; (D) Octanol/PBS partial coefficients of the two contrast probes.

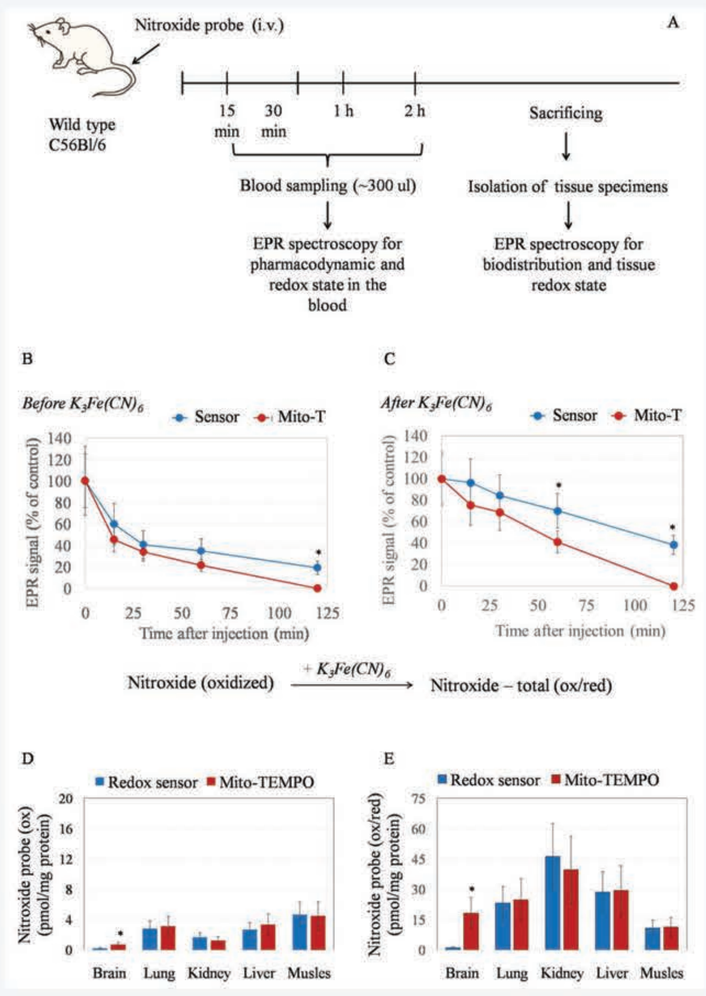
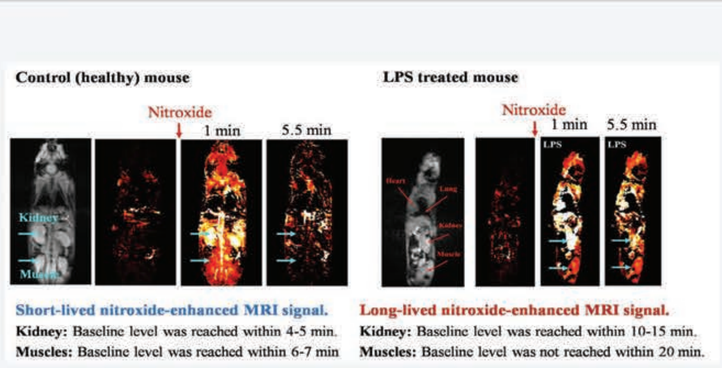


Figure 4. (A) Experimental design. Redox sensor and mito-TEMPO were injected intravenously in equal doses, corresponding to 10 μmol of nitroxide per mouse. (B, C) Electron paramagnetic resonance (EPR) signal dynamics of redox sensor and mito-TEMPO in the blood – before (B) and after (C) addition of potassium ferricyanide. Number of mice at each time-point – 6, with two parallel measurements per sample. *p<0.05 versus mito-TEMPO at the same time-point; (D) Amount of the oxidized form of redox sensor and mito-TEMPO in tissues; (E) Total amount of the redox sensor and mito-TEMPO in tissues. Number of mice in each group – 6, with two parallel measurements per sample. *p<0.05 versus RS in the same organ.



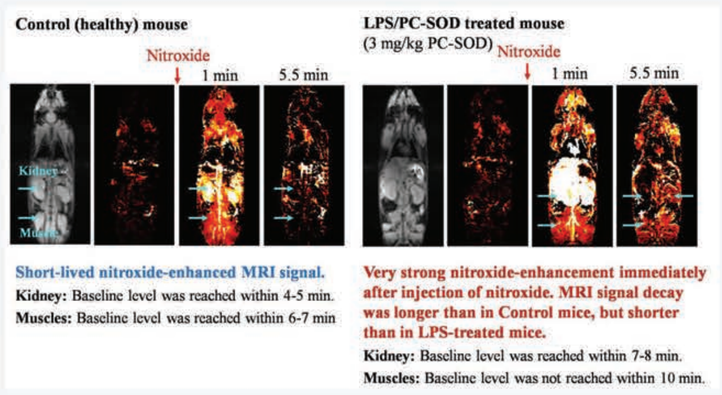
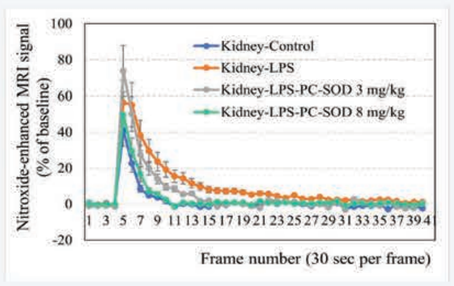


Figure 5. Dynamics of nitroxide-enhanced MRI signal in healthy, LPS-treated, and LPS/PC-SOD-treated mice after intravenous injection of redox sensor (T_1W gradient-echo MRI). Representative images (extracted nitroxide-enhanced MRI signal normalized to the baseline). Blue arrows indicate regions-of-interest (ROIs) – kidney and skeletal muscles. PC-SOD – superoxide dismutase (antioxidant enzyme and endogenous redox modulator)



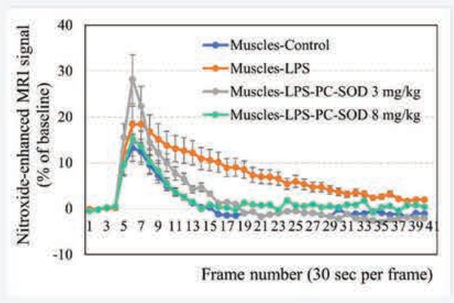


Figure 6. Dynamics of nitroxide-enhanced MRI signal in the kidney and skeletal muscles of healthy, LPS-treated, and LPS/PC-SOD-treated mice after intravenous injection of redox sensor (RS)(T_1W gradient-echo MRI): kinetic curves. Data are the means+/-SD from 7 mice in contral group and 5 mice in each other group.

CONCLUSION

RS is a biocompatible multi-spin probe suitable for EPR/MRI analyses of the redox state of tissues and body fluids in vitro and in vivo. It is characterized by the same EPR contrast and better MRI contrast compared to commercial mito-TEMPO. RS does not cause adverse side-effects when administered intravenously at the selected dose. RS circulates longer in the bloodstream than mito-TEMPO and is suitable for analyzing tissue redox state in blood, lung, liver, kidney and skeletal muscles. However, it does not appear to cross the blood-brain barrier and is inappropriate for redox imaging in the brain tissues, unlike mito-TEMPO. Regardless, RS may be suitable for redox imaging in the cerebral circulation, as well as for redox imaging of blood-brain barrier disruption. This assumption needs further verification, which is the goal of our future studies.

In addition, RS has a dual imaging modality – paramagnetic (in oxidized form) and optical (in reduced form) due to existance of quantum dot in the chemical structure, and can be applied simultaneously for EPR, MRI and fluorescence imaging. Sensor is also suitable for evaluation of the effects of drugs, such as PC-SOD, on tissue redox state and oxidative stress in vitro and in vivo.

Published articles:
Redox Reports 2023, 28(1), 2220531
Molecules 2025, 30(9), 1882

Acknowledgements:
This study was supported by the The European Union-Next-Generation EU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project SUMMIT BG-RRP-2.004-0008-C01.
We would like to thank Dr. I. Aoki, Dr. A. Sumiyoshi, and Dr. S. Shibata from the QST, Japan for their support in the MRI experiments.