ESR evidence of superoxide anion, hydroxyl radical and singlet oxygen generation during the photosensitization of PPME in HCT-116 cells

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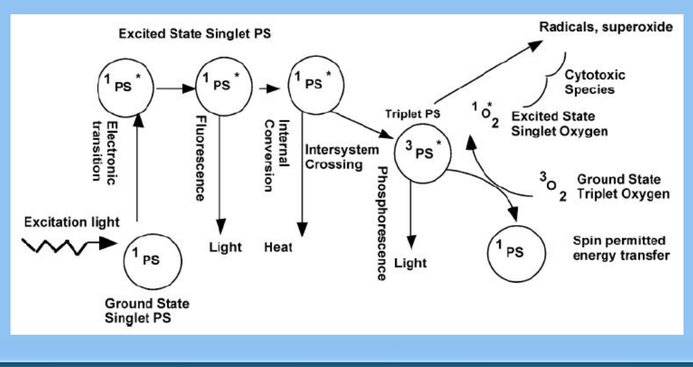
1. INTRODUCTION

Photodynamic therapy (PDT) appears to be a powerful technique against several common types of cancers (skin, prostate, esophagus,…). This treatment involves the combination of photoactive molecules (non-toxic in the dark), known as **photosensitizers** (PS), and light radiations belonging to the visible spectrum. The absorption of a photon by the PS, in the presence of oxygen, starts the creation of **reactive oxygen species** (ROS) that can damage cellular constituents leading to necrosis or apoptosis as final issue [1].

Photosensitizer molecules have a high probability of triplet state formation after excitation. PS in the triplet state can react with its environment via two competing pathways:

- Type-I photochemical reaction: **transfer of electrons** to solvent before ROS production;
- Type-II photochemical reaction: **transfer of energy** to oxygen to form the very reactive **singlet oxygen** [2].



2. EXPERIMENT

The photosensitizer used during our experiments is the *Pyropheophorbide-a methyl ester* (**PPME**). This 2nd generation PS is studying largely on cancerous cells *in vitro* for nearly a decade and seems to be a potent candidate for future clinical *in vivo* applications.

Our study has consisted of identifying and quantifying the ROS generation induced by light-activation of intracellular PPME.

Protocol:

5 µM of PPME has been incubated in HCT-116 cells (colon cancer cells) 20h before electron spin resonance (ESR) analysis. After excitation of cells at 633 nm (66kJ/m²), ESR spectrum were recorded.

3. ELECTRON SPIN RESONANCE

The ESP Brucker 300 used for the experiments

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

ESR allows the **quantification** and **qualification** of ROS produced by cells themselves, by oxidative stress (drug) or by therapeutic treatments (photodynamic therapy).

4. SPIN TRAPPING TECHNIQUE

The detection of short-lived free radicals by ESR must be indirect.

The **spin trapping** technique makes use of a diamagnetic compound, the **spin trap**, which reacts with a free radical creating a relatively stable ESR-observable radicalar component, the **spin adduct** [3].

$$R^{\cdot} + \quad spin \quad trap \rightarrow \quad spin \quad adduct^{\cdot}$$

Due to the intracellular localization of PPME, the majority of ROS are generated inside HCT-116. ESR experiments were thus performed using an intracellular located spin trap, **POBN** (*4-pyridyl 1-oxide-N-tert-butylnitrone*), in order to detect directly in situ the ROS production.

5. DETECTION OF HYDROXYL RADICALS

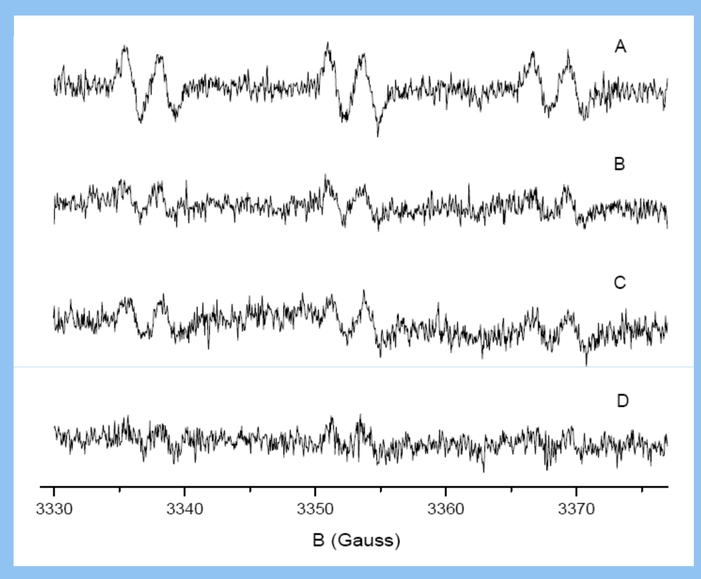
During the photosensitization of the PPME, hydroxyl radicals are produced via the Fenton reaction:

$$O_2^{\cdot -} + Fe^{3+} \rightarrow {}^3O_2 + Fe^{2+}$$
$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\cdot}$$

RESULTS: Irradiation of PPME in HCT-116 cells in the presence of POBN spin trap and ethanol (EtOH) scavenger leads to the apparition of the ESR spectrum characteristics of POBN/ethoxy adduct.

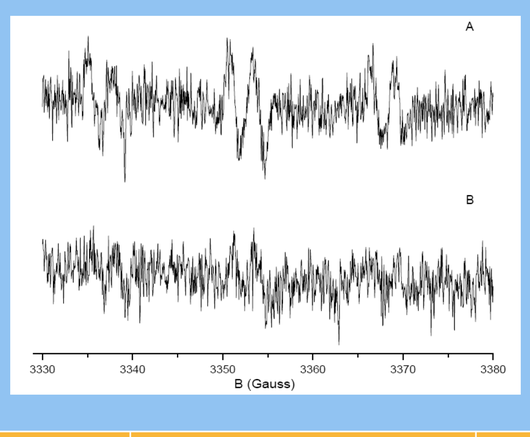
Chemical substances	Property	Reduction of the signal
Catalase (CAT)	Hydrogen peroxide quencher	30%
Superoxide dismutase (SOD)	Superoxide anion quencher	30%
Desferroxamine	Iron chelator	20%

ESR spectrum of intracellular photoexcited PPME 5µM
control (A)
with catalase (B)
with SOD (C)
with desferroxamine (D)



6.DETECTION OF SINGLET OXYGEN

RESULTS:



ESR spectrum of intracellular photoexcited PPME 5µM
(A) control
(B) with DABCO

Chemical substances	Property	Reduction of the signal
DABCO	Singlet oxygen quencher	70%

7. CONCLUSIONS

•It was shown using ESR associated with spin trapping technique that photoexcited PPME is able to generate **superoxide anion**, **hydroxyl radical** and **singlet oxygen**.

•Production of POBN/ethoxy was partly inhibited by SOD, catalase and desferroxamine, suggesting that the **Fenton reaction** is the way of **formation of hydroxyl radical**.

•The complementary inhibition of POBN/ethoxy production by DABCO is consistent with **singlet oxygen generation** by PPME. Furthermore, it makes appear the possibility of singlet oxygen detection by POBN and EtOH.

•All our results seem to indicate that PPME is a **30%/70%** type I/ type II sensitizer in HCT 116 cells.

REFERENCES

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[3] C. Anderson Evans, «Spin trapping», in *Aldrichimica Acta* (1979) 12, 2