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 photosensitive molecules (non-toxic in the dark), known as photosensitizer (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.

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,

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

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.

3. ELECTRON SPIN RESONANCE

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

ESR spectrum of intracellular photoexcited PPME 5µM

The ESP Brucker 300 used for the experiments

4. SPIN TRAPPING TECHNIQUE

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

Due to the intracellular localization of PPME, the majority of ROS are generated inside HCT-116. ESR experiments were thus performed using an intracellular localized spin trap, POBN (4-phenyl-1-oxide-2-nitroso-1,2-dihydro-1,2-dithiin) 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:

\[
\text{O}_2^{-} + \text{Fe}^{2+} \rightarrow \text{Fe}^{3+} + \text{O}_2 + \text{H}_2\text{O}
\]

\[
\text{Fe}^{3+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{2+} + \text{OH}^{-} + \text{OH}^{\cdot}
\]

**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.

6. DETECTION OF SINGLET OXYGEN

**RESULTS:**

<table>
<thead>
<tr>
<th>Chemical substance</th>
<th>Property</th>
<th>Extinction at the signal</th>
</tr>
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<tbody>
<tr>
<td>POBN</td>
<td>Singlet oxygen quencher</td>
<td>70%</td>
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7. CONCLUSIONS

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

- Production of POBN/ethoxy was partly inhibited by SOD, catalase and deferoxamine, suggesting that the reaction is the way of formation of hydroxyl radical.

- The complementary inhibition of POBN/ethoxy production by DABCO is consistent with singlet oxygen detection by POBN and ETOH.

- All our results seem to indicate that PPME is a type I/II sensitizer in HCT 116 cells.

REFERENCES