

**TITLE:** PORPHYRINS: POWERFUL PHOTOSENSITIZERS AGAINST *LEPTOSPIRA INTERROGANS*

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**ABSTRACT:**

The porphyrin *meso*-tetramethylpyridyl (TMPyP) and its metallized form with zinc (ZnTMPyP) are cationic photosensitizers that have been extensively tested in the photodynamic inactivation (PDI) technique against infectious agents since they are notable for not causing natural microbial resistance. The method combines a photosensitizer (PS), light and molecular oxygen and results in the formation of reactive oxygen species (ROS), which at high levels induce cell death. *Leptospira interrogans* is a Gram-negative aerobic spirochete that can be disseminated through urine, water, venereal and transplacental routes. It is responsible for the zoonosis called leptospirosis, which brings considerable economic losses. In order to evaluate the effect of porphyrins on the PDI of *Leptospira interrogans* in vitro, the PSs TMPyP and ZnTMPyP were used. The culture was incubated with each PS (10 $\mu$ M) for 1h without light; diluted in EMJH medium (1:4) and then quantified in a spectrophotometer (OD of 600 nm). The samples were placed in a microtiter plate and irradiated at an intensity of 180mW/cm<sup>2</sup> for 30min using a 500W halogen lamp (470 to 750nm). The controls were: culture without PS/irradiation; culture without PS with irradiation; culture with PS without irradiation. An aliquot of each sample (irradiated and controls) was diluted in EMJH medium (1:4), incubated at 29 °C for seven days and evaluated by dark field microscopy and spectrophotometer reading. No significant difference was observed among the controls samples, indicating that the irradiation by itself, as well as in the absence of PS, did not affect bacterial growth. The samples treated with the irradiated PSs showed significant difference when initial and final ODs values were compared. Although no significant difference was observed between the porphyrins, a greater cell destruction was noted in the sample irradiated with ZnTMPyP, possibly due to increase in the interaction between the metal in the central ring of the porphyrin and the bacterial membrane. Being both cationic, we believe that the porphyrins used in this work have a great electrostatic interaction with the negative charges displayed by the phospholipids on the cell membrane surface, increasing the photodynamic damage. Therefore, both porphyrins TMPyP and ZnTMPyP can be used as a powerful tool for the control of *Leptospira interrogans*.

**Keywords:** leptospirosis, PDI, photosensitizer, porphyrins

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