

Poster

In vitro evaluation of the production of reactive oxygen species in silver metallophosphazenes



Gil López, Ana (1), Batanero Mantero, Ana (1), Maisanaba Hernandez, Sara (1*), Llana-Ruiz Cabello, María (1), Jiménez Villar, Josefina (2) y Repetto Khun, Guillermo (1*)

(1) Departamento de Biología Molecular e Ingeniería Bioquímica. Área de toxicología. Universidad Pablo de Olavide. Ctra. Utrera, km1 – 41013 Sevilla.

(2) Departamento de Química Inorgánica, Facultad de Ciencias, Instituto de Síntesis Química y Catálisis Homogénea, Universidad de Zaragoza-CSIC, 50009 Zaragoza.

Tutor académico: Repetto Kuhn, Guillermo; Maisanaba Hernández, Sara.

Keywords: In vitro assays; Oxidative stress; Phosphazenes; Silver.

ABSTRACT

Motivation: Phosphazenes (FZ) are polymers that have been shown to have a high therapeutic potential in recent years (Andrianov and Allcock, 2018). Among its derivatives, metallophosphazenes should be highlighted due to the interest in the biological activity of metal complexes (Gascón et al., 2020). Likewise, the estimation of the toxic risk of a substance is a complex process in which the risks and benefits for organisms or populations are related (Repetto y Repetto 2009). In vitro models of the cell culture type allow studying the toxicity of xenobiotics at the systemic, cellular and molecular level (Meseguer, 2016).

Methods: In the present work, the oxidative stress induction of two silver-FZ complexes was evaluated by studying the generation of reactive oxygen species (ROS) (Meseguer, 2016) in the MCF-7 cell line. The intracellular formation of ROS was determined fluorimetrically with the reagent 2', 7'-dichlorodifluorescein 3', 6'-diacetate (Hempel et al., 1999); using tert-butyl hydroperoxide and hydrogen peroxide as positive controls. The concentrations used were the EC50 and its sub-concentrations, being for compound 1: 2.34 μ M, 1.17 μ M and 0.585 μ M; and for compound 2, 1.60 μ M, 0.80 μ M and 0.40 μ M. These concentrations were determined using the alamar blue and neutral red cytotoxicity tests. ROS production was measured every 30 min, from time 0 to 120 min. Statistical significance between concentrations and the control was determined by Dunnett's Multiple Comparison Test, using GraphPad Software.

Results: Compound 1 showed the disappearance of ROS observed ($t = 0$ min $p < 0.01$) throughout the test time, observing non-significant differences with respect to the control at the end of the same ($t = 120$ min). However, compound 2 maintained a statistically significant difference throughout the experiment for all concentrations, except for the maximum concentration tested (1.60 μ M), which shows a decrease at the end time ($t = 120$ min).

Conclusions: In view of the results obtained, it is observed that the cells exposed to compound 1 seem to recover over time from the initial induction of ROS. Compound 2 induces ROS in MCF-7 cells. This difference in behavior could be related to the different proportion of silver present in both compounds, of one and two molecules, respectively. It is necessary to carry out a more exhaustive evaluation of the oxidative profile of these compounds, in order to determine if antioxidant defense mechanisms could be active.

REFERENCES

- Andrianov K. A., Allcock R. H. (2018). Polyphosphazenes in biomedicine, engineering and pioneering synthesis.
- Gascón, E., et al. (2020). (Amino)cyclophosphazenes as Multisite Ligands for the Synthesis of Antitumoral and Antibacterial Silver(I) Complexes. *Inorg Chem.* 59(4), 2464-2483.
- Hempel S. L. et al. (1999) Dihydrofluorescein diacetate is superior for detecting intracellular oxidants: comparison with 2',7'-dichlorodihydrofluorescein diacetate, 5 (and 6)- carboxy-2',7'- dichlorodihydrofluorescein diacetate and dihydrorhodamine 123. *Free Radi Bio Med.* 27, 146-159.
- Meseguer F. A. (2016). Evaluación toxicológica in vitro de los efectos producidos por cadmio, plomo y selenio en células renales y hepáticas, a concentraciones encontradas en tres especies de pingüino de la Península Antártica e islas asociadas. PhD, Thesis, Escuela Internacional de Doctorado, Univ de Murcia, España.
- Repetto J. M., Repetto K. G. (2009) *Toxicología fundamental*. 4ª Ed. Editorial Diaz de Santos.