

**European Society of Photobiology
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Report of Monica Camerin

I visited Dr. Angeles Villanueva's laboratory in Madrid for two weeks, in September (20/09/04 – 03 /10/04). I co-worked with Dr. Santi Rello Varona in the frame of the project "Photothermal sensitization of Biological Systems: Mechanisms of actions"

Methods

During this time suitable prepared samples of B78H1 cells (melanoma amelanotic cells) were analysed by SEM (scanning electron microscope), as well as by optical microscope. For the observation at the SEM the cells were incubated with Pd(OBu)₈Nc using two incubation times (18 and 48 h) and irradiated for different time intervals. Cells incubated at two incubation times with different concentrations (7.7 μM, 15 μM) were observed at the optical microscope for investigating the localization of Pt(OBu)₈Nc in the subcellular compartments. Studies of fluorescence microscopy were performed for the identification of the drug distribution among various cellular organelles including Golgi apparatus, microtubules, lysosomes in this type of cells.

Results

The SEM observation can show the type of damage that the photothermal sensitization induced in the cells. In this case we obtained images showing the photoinduced leakage of cytoplasmic material from the irradiated cells and the consequent formation of deep cavities in the cells. So the mechanism of death for the photothermal treatment is not apoptosis but the extensive morphological changes caused by the rapid and sudden water evaporation due to the shock heating. The observations at the optical microscope clarify the distribution of naphthalocyanine in the cell. The type of distribution is important because, theoretically, the presence of aggregated drug allows the possibility of absorption of more than one photon in the same volume before the first temperature spike suffered much dissipation, and increased the damage at the cells. Besides, the type of damage depends also on the localization of the photothermal sensitizer. Our substances are not fluorescent and so, in order to obtain reliable indications about the pattern of their subcellular distribution, it is necessary to treat the naphthalocyanine – incubated cells with fluorescent probes which are specific for determined organelles and superimpose the fluorescence image on the optical image.

Conclusions

The obtained data are very important for the development of our project. The Villanueva's laboratory has the knowledge and the scientific instrumentation to advance the photothermal studies. In particular, we focused our attention on the photosensitizer microenvironment, the sites of sub-cellular distribution and the correlation of the type of subcellular distribution with the photothermal destruction of cells.

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