PHOTOSENSITIZATION OF CHINESE HAMSTER CELLS BY A CHLOROPHYLL DERIVATIVE (PHEOPHORBIDE a): RELATIONSHIP TO CELL LOCALIZATION, DISRUPTION AND INACTIVATION

Mihoko SAITO

Department of Radiation Biology,
Faculty of Veterinary Medicine,
Hokkaido University, Sapporo 060, Japan

Pheophorbide a (PPa) ingestion is a cause of dietary photosensitization in animals. The present study was made to provide evidence that PPa sensitized cultured mammalian cells to visible light with wavelengths over 600 nm. Chinese hamster fibroblasts, line V79-B310H, were used. The light fluence-response curves for phototoxicity of PPa were constructed using the colony-forming ability of cells as an end point. Under the experimental conditions used, a fluence threshold was observed around 5KJ/m^2. Since the inactivation of cells by photosensitization was not affected by glycerol (OH radical scavenger) but drastically inhibited by N_3^- (singlet oxygen quencher), it was concluded that Type II photodynamic action was responsible for the PPa phototxicity.

When the intracellular distribution of PPa was observed using laser scan microscopy, PPa fluorescence was found to be localized in the plasma membrane, cytoplasm and nuclear membrane, and not in the nucleus. When the morphological alterations were observed 2 hours after photosensitization with the differential interference microscope, it was found that the cells tended to become spherical and intracellular vacuoles had formed. A number of cultured cells started to degenerate 2 hours after photosensitization. Almost 100% of cells were completely disrupted 20 hours after photosensitization.

The photo-induced changes in DNA integrity were also investigated using the alkaline-filter elution and pulse-field gel electrophoretic techniques. Results showed that PPa caused not only single-strand breaks but also double-strand breaks of DNA. This suggests that DNA has no connection with the photo-induced inactivation of Chinese hamster V79 cells by PPa.

From these results it was concluded that massive damage to cytoplasm including plasma membranes and nuclear membranes led to cell death after PPa photosensitization.