Laser-Excited Photochemical Effect on Biological Cells Containing Pheophorbide a II. Time-Resolved Measurements of Fluorescence Intensity Distribution in a Cancer Cell with Laser-Excited Fluorescence Microscope System

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In the previous paper I [1], we have reported the direct spectroscopic detection of the singlet molecular oxygen ($^{1}$O$_{2}$) in a photosensitive system containing pheophorbide a, which is one of the decomposition products from chlorophyll [2], and the remarkable evidence of laser-induced photochemical effect on cultured cancer cells uptaken this new dye. It was found experimentally that pheophorbide a results in more effectively the photochemical action in cultured esophagus cancer cells (TE-2) than hematoporphyrin derivative (HpD) and hematoporphyrin dihydrochloride (Hp.2HCl) under the condition of nearly same concentrations and same irradiation time of Ar ion laser output. This fact leads to the inference of this new dye to be a good candidate for photoradiation therapy (PRT) in the treatment of certain tumors, based on the cell damage by lipid peroxidation through efficient production of $^{1}$O$_{2}$. In this paper II, we describe some results of further study of the laser photosensitized effect of pheophorbide a on individual cancer cells using a very high sensitive laser-excited fluorescence microscope system which was designed and built in our laboratory.

As reported already [3,4], our new system for laser-excited fluorescence microscopy possesses excellent sensitivity and spatial resolution for a single biological cell by the use of an ultra-high sensitive image camera and digital image processing technique for the three dimensional display of intensity distribution. An Ar ion laser oscillating at 488.0 nm for the excitation and a Nikon XF microscope were employed for the present experiment. The laser beam after passing through a diffuser for the uniform irradiation was directed to a sample on a slide glass by a dichroic mirror in the microscope. The detection of the fluorescence image of the sample was performed by a SIT camera (Hamamatsu C1000-18) and its image signals were digitized by an image processor to store the spatially resolved fluorescence intensity in a frame memory. Both digitized and directly detected images by the SIT camera were displayed on TV monitors, and the data in the frame memory were transferred to a floppy disk by a microcomputer for necessary image processings. It is to be mentioned that laser output power of a few mW on the diffuser surface was enough for the detection of fluorescence images.
within one second in our present experiment.

PBS solution containing 0.8 % albumin was employed as the solvent of pheophorbide a to obtain the concentration of $5 \times 10^{-5}$ mol/l. Cultured esophagus cancer cells (TE-2) was cultivated in this solution for one hour.

Figure 1 shows a series of three dimensional graphic displays of time-resolved fluorescence intensity distribution across a TE-2 cancer in every six minute interval after the laser irradiation, measured by our laser-excited fluorescence ultra-high sensitive microscope system. The laser power density on the sample surface was estimated to be approximately 0.11 mW/cm². It is quantitatively seen that the fluorescence intensity is weak at the central portion of the cell, while it is strong around it. This result indicates that pheophorbide a is mainly uptaken in cytoplasmic membrane of this cancer cell, which was more remarkable compared with HpD and Hp.2HCl uptaken in the same cell. From Fig. 1, we can clearly understand that the fluorescence intensity decreases with time and its distribution expands gradually by the photochemical process caused by the uptake of pheophorbide a. This feature was found to coincide with the visual observation of temporal change in the cell damage after the laser irradiation, through conventional microscope images displayed on a TV monitor in our system.

Further investigations are in progress in our laboratory to clarify performance and capability of pheophorbide a in the PRT for various kinds of cancer cells, and the results will be reported successively.