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The Photocytotoxicity of Talaporfin Used in Photodynamic Therapy

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The accumulation of photo-sensitizer Talaporfin (another name is NPe6) in cancer cells after incubating in NPe6 solution has been investigated. It was confirmed that Talaporfin can only kill cancer cells selectively by photo-irradiation at certain wavelengths. In this experiment, the WFB (rat fetus fibroblast) and W31 (the malignant transformed cell from WFB) were used as normal and cancer cell. The measurement of the optical absorbance of Talaporfin within the cell confirmed that the Talaporfin was accumulated in cancer cells more than in normal cells. Fluorescence image of cancer cell after incubating in Talaporfin solution was measured with a fluorescence microspectrometer. For comparison the standard white light image of cancer cell was also taken. A semiconductor laser was used as PDT (photodynamic therapy) optical source to compare the photocytotoxicity effect of the W31 and WFB cell. The viability of the cells was assessed by use Trypan Blue Stain. It was clarified that Talaporfin could be accumulated in cancer cells more than in normal cells and lead to cancer cell death selectively after irradiation.

Keywords: absorption spectral; cancer cells; fluorescence image; normal cells; photocytotoxicity effect; photodynamic therapy; semiconductor laser; Talaporfin; viability of cells

1. INTRODUCTION

Although modern medical treatments have been greatly advanced, cancer is still listed at the top of the mortality rate. Along with the development of endoscopic imaging system and surgical treatment

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technique, the diagnosis and treatment level have improved significantly. However, early diagnosis of cancer and treatment of most spreading cancer are not satisfying. The photodynamic diagnosis (PDD) and the photodynamic therapy (PDT) which combine the photo-sensitize and laser irradiation at special wavelength have been the subject of intense studies [1–5].

The procedure of PDT is: Firstly, the photo-sensitize is injected into the vein of the patient. Photosensitize penetrated into both cancer and normal cells. But after several hours, photosensitize in normal cells is excreted due to metabolism, and the concentration in cancer cell is still high because of the affinity of the photosensitize with tumor tissue. Then, the tumor tissue is irradiated under laser beam, which cause PDT effect. The PDT can do damage to cancer cells. In this paper, the absorption spectrum and the fluorescence image of cells were recorded and analyzed. The quantity of the photosensitize accumulation in cancer cell was investigated. The survival rates of cancer cell and normal cell were also measured and these results testified the photocytotoxicity of photosensitizer effect on the cancer cell and the normal cell.

2. MATERIALS AND METHODS

2.1. Photosensitizer Talaporfin

The Talaporfin, with a molecular weight of 799.69, has a chlorin structure with a double bond in the D ring of the tetra-pyrrole ring growing one detachment. Its structure consists of one aspartic acid salt attached on the side chain in the carbon 15 position of this ring due to the amid combination (Fig. 1). In phosphate buffer saline solution at pH = 7.4, the Talaporfin has absorption peak at 398 nm (the Soret band) 502 nm, 530 nm, 620 nm and 654 nm (the Q bands) [6,7].

The absorption band of the tetra-pyrrole ring accumulated in the tumor is bathochromic shifted to longer wavelength about 10 nm. Tetra-pyrrole ring is conformational changed by the combination for the substitution with the living molecule. The $\pi \rightarrow \pi^*$ transition of the pyrrole ring causes a red spectral shift due to the approach of the polar solvent and the interaction with the substitution group.

The Q band of the Tetra-pyrrole ring of Talaporfin have an absorption peak at 664 nm. When excitation is given in the absorption wavelength (398 nm) of the Soret band of the Tetra-pyrrole ring, Talaporfin shows fluorescence spectrum peak at 662 nm. When excitation is given in the absorption wavelength of 664 nm, the characteristic fluorescence spectrum of Talaporfin shows peak at 672 nm [8].

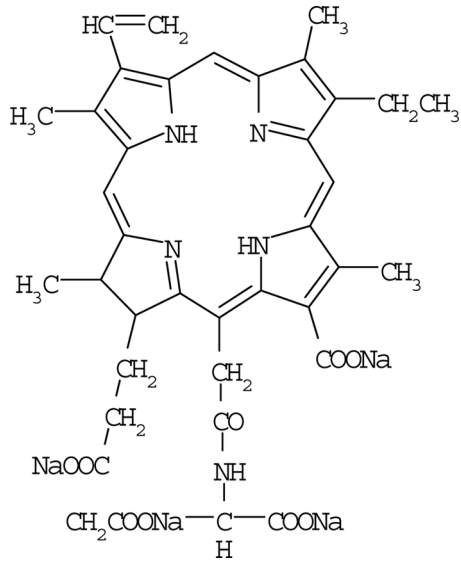

FIGURE 1 Chemical structure of Talaporfin.

2.2. Cell and Cell Culture

In this study, W31 cell (cancer cells) and WFB cell (normal cells) are used. WFB cell (normal cells) is rat fetus-derived fibroblast. W31 cell (cancer cells) is established by transformation of fetal fibroblastic WFB cells with H-ras oncogene.

These cells were originally provided by Sapporo Medical University. We cultivated these cells in DMEM (Sigma-Aldrich) supplemented with 10% FBS (GIBCO Industries, Inc.).

2.3. Experimental Setup

The absorption spectrum was measured using a spectrometer (Shimatsu, UV-2400PC). The image of cancer cell was recorded by a microscope with dual mode cooled CCD camera (Hamamatsu, OLYPUS IX70). Figure 2 shows the experimental setup for the study of the photocytotoxicity effect. The wavelength of the diode laser [9] is centered at 664 nm and the output power is 35 mW. The laser beam was coupled into a 400 μm-diameter fiber, and collimated with a 50 nm-focal-length lens. In order to illuminate cover the whole dish, a 35 mm-focal-length lens was placed before the culture dish at the distance of 35 mm.

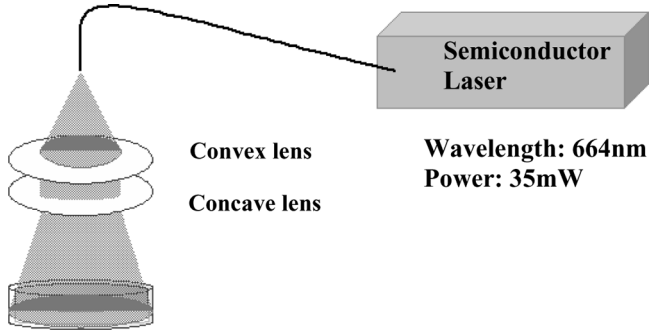

FIGURE 2 Experimental setup for the study of the photocytotoxicity effect.

3. RESULTS AND DISCUSSION

3.1. Absorption Spectrum of Talaporfin in Cell

The absorption spectrum of both normal and cancer cells after incubating in Talaporfin solution was measured for investigating the effective wavelength for the photocytotoxicity effect. The concentration of Talaporfin is 5.0×10^{-5} M. After 30-minute incubation, the penetrated cells were centrifugalized, and the photosensitizer Talaporfin outside the cell was rinsed by using PBS. The absorption spectra of both cancer and normal cell were measured and gave out in Figure 3. If the cancer cells and normal cells were not incubated in Talaporfin solution, no absorption spectrum peak was observed. The result gave out in Figures 3(a) and 3(b). The absorption band from 390 to 420 nm and band from 640 to 680 nm (Figures 3(c) and 3(d)) were obtained from both cancer and normal penetrated cell. With the same concentration and irradiation duration, the absorption peaks of cancer cell are higher than that of normal cell. This is because the cancer cell has more coenzyme NADH, and absorbs more Talaporfin. From this phenomenon, we can conclude that the photosensitizer accumulation in cancer cell is more than normal cell. From experimental absorption spectra, the laser wavelengths for PDT can select at 400 nm and 640 nm. Because the wavelength at 400 nm is also the absorption band for hemoglobin in human blood, so we choose the 664 nm wavelength for stimulation in our cell killing experiment.

3.2. The Fluorescence Images of the Cancer Cell after Talaporfin Penetrated

The fluorescence images of the cancer and normal cell after penetrated photosensitizer Talaporfin were recorded using microscope with dual mode cooled ICCD camera (Hamamatsu, OLYPUS IX70). The solution

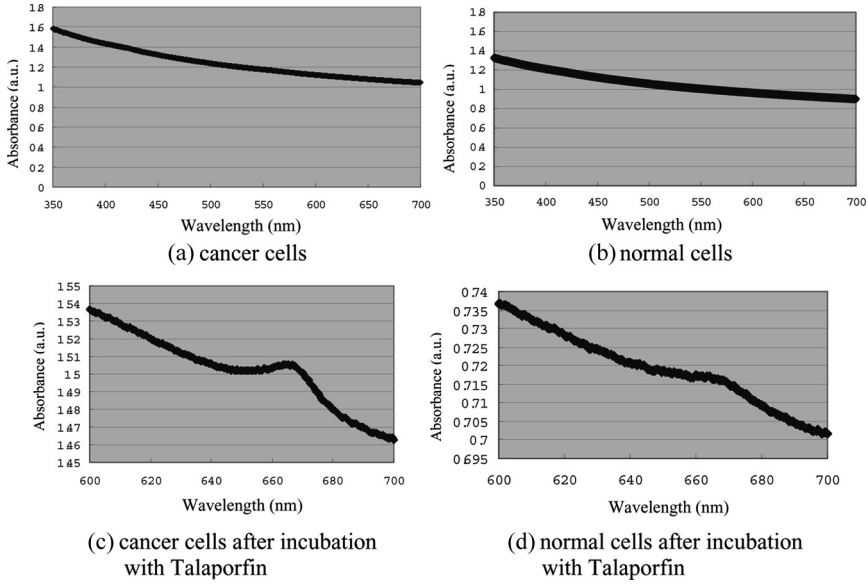

FIGURE 3 Absorption spectra of the cells.

concentration of Talaporfin is 1.0×10^{-4} M. The incubation duration is 30 minutes. Figure 4(b) is the fluorescence image of Talaporfin penetrated cancer cells. The image fluorescence peak is at 670 nm, while the exciting wavelength is at 400 nm. As reference, the white image of penetrated Talaporfin cancer cells was also debilitated shown in Figure 4(a). Comparing 4(a) and 4(b), it is clear that Talaporfin has already entered inside the cells. Under same experiment condition, the white image and fluorescence images of normal cell were obtained (shown in Figures 4(c) and (d)). The fluorescence intensity from cancer cells (see 4(b) is stronger than that from normal cell (see 4(d)). It proves that more photosensitizer is accumulated in cancer cell. Figure 4(e) is the fluorescence image of Talaporfin penetrated cancer cells which under irradiated by diode laser for 15 minutes. The intensity of the fluorescence is decreased obviously. This photo bleaching phenomena is caused by the cleavage of tetrapyrrole compounds due to laser irradiation.

3.3. Normal and Cancer Cell Photocytotoxicity Effect with PDT

The PDT killing cell experiment was carried out using a diode laser PDT system. The photosensitizer Talaporfin solution was injected into

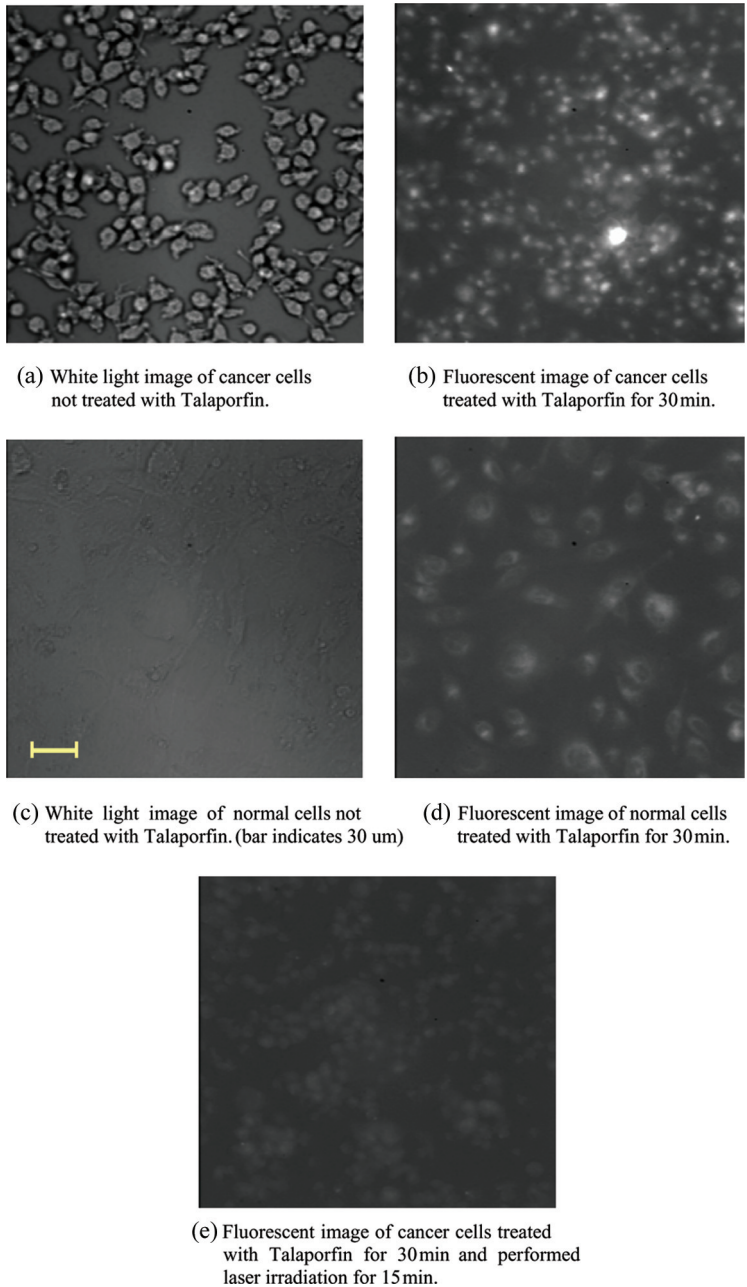

FIGURE 4 Image of cancer cells and normal cells after incubation with Talaporfin.

35-mm culture dish. Two different Talaporfin concentration of 5.0×10^{-6} M and 2.5×10^{-6} M were used. The normal cells and cancer cells were seeded into the dish respectively. After action of photosensitizer with cell for 30 minutes, the cultured cells were performed PDT treatment with different light doses delivered by a diode laser for 5, 10, 20, 25 and 30 minutes, respectively. After PDT treatment, the samples were centrifugalized twice. Then, the survival rate of the cells was analyzed by the Trypan Blue Stain assay. The photocytotoxicity effects on both cancer cells and normal cells were measured 5 times under two solutions concentration. The experimental results are shown in Figure 5. The bar graph is the average value of 5-time measurement, while the error bar is the error of average value. After 15-minute illumination, the survival rate of normal cell (WFB) was 55.8%, while that of the cancer cell (W31) was 36.5%, while the concentration of Talaporfin solution was 2.5×10^{-6} M. The survival rate of normal cell (WFB) and cancer cell (W31) were 53.6% and 35.9% respectively, while the concentration of Talaporfin solution was increased to 5.0×10^{-6} M and the illumination time is not changed. For these two concentrations, the survival rate of normal cell was 1.5 times higher than that of cancer cell. If rise the illumination time to 20 minutes, and the concentration solution was 2.5×10^{-6} M, the survival rate of normal cell was 49.4%, and that of cancer cell was 33.2%. If rise the solution concentration to 5.0×10^{-6} M and the irradiation duration is still 20 minutes, the survival rate of normal cell and cancer cell was 42.3%

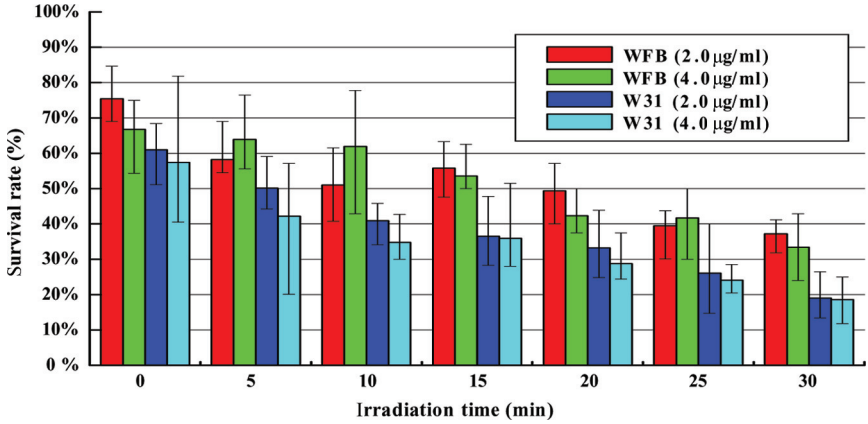

FIGURE 5 Survival rate after PDT for different Talaporfin concentration. The bars in the figure represent mean values ± standard deviations. The data shown are averaged values for five measurements.

and 28.8% respectively. The survival rate of normal cell was not multiple higher than that of cancer cell. For the irradiation duration was up to 30 minutes and the solution concentration was 2.5×10^{-6} M, the survival rate of normal cell was 37.2%, and that of cancer cell was 19%. The survival rate of normal cell was 2 times higher than that of cancer cell. For 30-minute irradiation and concentration 5.0×10^{-6} M, the survival rate of normal cell was 33.4%, and that of cancer cell was 18.6%. The survival rate of normal cell was 1.8 times higher than that of cancer cell. If decrease the concentration to 2.5×10^{-6} M, the survival rate of normal cell was almost not change, when the laser irradiation duration varied from 5 to 15 minutes. It seems the concentration of Talaporfin is too lower to penetrate to the normal cell. For the 5.0×10^{-6} M concentration, the survival rate of normal cell increased gradually along with the increasing irradiation duration, while the survival rate of cancer cell increased significantly. Our experiments testified that the photocytotoxicity effect of Talaporfin on cancer cell was much higher than that on normal cell.

4. CONCLUSION

The experimental data proved the photosensitize Talaporfin can penetrate into normal and cancer cell of the rat, and exist in cell. The absorption spectra and the fluorescence image of the normal and cancer cell with Talaporfin were measured. Furthermore, the PDT was carried out with different irradiation duration from 5 to 30 minutes at 5-minute intervals, under the same culture duration (30 minutes), same concentration (2.5×10^{-6} M, 5.0×10^{-6} M) and same laser beam flux. The results showed that the survival rate of cancer cell is much lower than that of normal cell. It testified that the photocytotoxicity effect of Talaporfin on cancer cell is much higher than that on normal cell. It also clarified the high accumulation of Talaporfin in cancer cell.

REFERENCES

[1] Hayata, Y., Kato, H., Konaka, C., *et al*. (1982). *Chest*, *81*, 269–277.
[2] Sheyhedin, I., Okunaka, T., Kato, T., Yamamoto, Y., *et al*. (2000). *Laser Surg Med.*, *26*(19), 83–89.
[3] Li, L., Aizawa, K., Kodama, K., & Minamitani, H. (2001). *Bioimages*, *9*(3–4), 107–116.
[4] Peng, Q., Warloe, T., Moan, J., *et al*. (2001). *Cancer Research, 61*, 5824–5832.
[5] Xiao, Z., Hansen, C. B., Theresa, *et al*. (2005). *J. Pharm. Pharmaceut. Sci.*, *8*(3), 536–543.

[6] Aizawa, K., Okunaka, T., Ohtani, T., *et al*. (1987). *J. Photochem. Photobiol.*, *46*(5), 789–793.
[7] Tsuchida, T., Aizawa, K., Konaka, C., & Kato, H. (1993). *Lasers in the Life Sciences*, *5*(3), 155–164.
[8] Li, L., Kodama, K., Saito, K., & Aizawa, K. (2002). *J. Photochem. Photobiol. B:Biol.*, *67*, 51–56.
[9] Katsumi, T., Aizawa, K., Okunaka, T., Kuroiwa, Y., *et al*. (1994). *Jpn. J. Cancer Res.*, *85*, 1165–1170.