김일성종합대학학보
(자연과학)
주체103(2014)년 제60권 제6호

JOURNAL OF KIM IL SUNG UNIVERSITY
(NATURAL SCIENCE)
Vol. 60 No. 6 JUCHE 103(2014).

Characteristics of Integration of Pheophytin and Pheophorbide in Cancer Cells

Han Ji Hyon, Nam Chang Yon

Porphyrin compounds are widely in use for diagnosis and treatment of incurable diseases including tumor because of their capacity to effectively photosensitize and fluorescence and, in particular, to be integrated into growing cells including oncocytes, and research into the ways of increasing their effects are underway on a large scale. [3, 4]

This paper is on integrated characteristics of pheophytin and pheophorbide in cancer cells.

1. Materials and Methods

Materials. Faeces of mulberry siliworms, collected and dried in shadow by sericulturists of Ryuso Vegetable Cooperative Farm in September, 2012.

Used as normal cells are epithelial cells and as cancer cells, uterine cancer cells.

Apparatuses; UV-Vis spectrometer "UV-2201" was used to identify pheophytin and pheophorbide, and fluorescence microscope "NEVOR 2" to observe cells.

Extraction and separation of chlorophyll; by previous method [1].

Making of pheophytin. Pheophytin was made by adding 5% HCl into the same volume of ethanol solution of chlorophyll, leaving it in darkness for one night, filtering it with filter paper and washing with distilled water until the pH became neutral. It was confirmed by measuring its UV-Vis absorbance spectre.

Composing pheophorbide. Pheophorbide was composed by putting 20% HCl into the same volume of ethanol solution of pheophytin, leaving it in darkness for 1 hour, filtering it with filter paper and washing it with distilled water until the pH became neutral. It was confirmed by measuring its UV-Vis absorbance spectre.

Characteristics of integration of pheophytin and pheophorbide in cells. Epithelial cells of villi and cancer cells were put into IMDM culture fluid at the concentration of 1×10^6/mL into which various amounts of pheophytin or pheophorbide were added to make a number of experimental culture fluid and cultivate the cells in them. The cultured cell fluid was put on the slide by one drop using a syringe and observed with fluorescence microscope, taken a photo, and its intensity of fluorescence was analyzed by a computer. The fluorescence intensity was calculated by averaging the R(red), G(green) and B(blue) values on Photoshop.

2. Results and Consideration

First, study was made of the characteristics of integration of pheophytin and pheophorbide in epithelial cells of villi.

2 % of pheophytin and pheophorbide ethanol solutions, the concentrations of which are 5mg/mL [2], were added respectively into cell culture fluid and their fluorescence intensities were measured according to culturing time (Fig. 1).

As shown in Fig. 1, the fluorescence intensity of pheophytin and pheophorbide increased rapidly for 3 hours, and then decreased to naught after 8 hours. This indicates that the concentration of pheophytin and pheophorbide reaches its maximum within about 3 hours in epithelial cells of villi and the Stewartia *koreanna* Nak. duration in cells is about 8 hours.

Next, study was made of the characteristics of integration of pheophytin and pheophorbide in cancer cells.

Pheophytin or pheophorbide was added into cancer cells and the change of fluorescence intensity according to culture time was measured (Fig. 2).

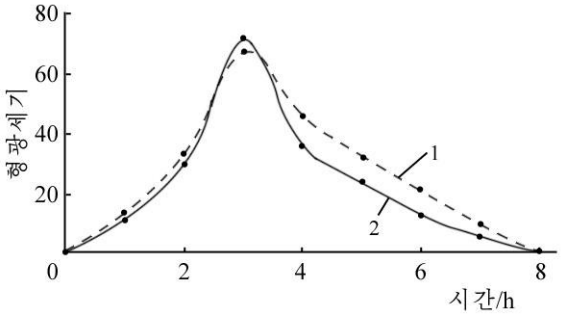

Fig. 1. change of fluorescence intensity in epithelial cells of villi with culture time
1－pheophytin, 2－pheophorbide

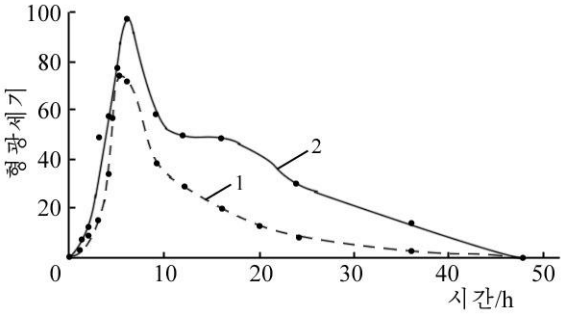

Fig. 2. change of fluorescence intensity in cancer cells with culture time
1－pheophytin, 2－pheophorbide

As is shown in Fig. 2, the fluorescence intensity increased rapidly for 6 hours after addition of pheophorbide in cancer cells followed by gradual decrease down to no fluorescence after 48 hours. This indicates that the concentration of pheophorbide reaches its maximum within about 6 hours and its duration is 48 hours. Similar results were got for pheophytin, but the maximal fluorescence intensity was lower than that of pheophorbide.

The characteristics of integration of pheophytin and pheophorbide in cancer cells and epithelial cells of villi were summarized as in tables 1 and 2.

Table 1. Characteristics of integration of pheophytin in cancer cells and normal cells

Type of cells	Maximal fluorescence intensity	Time to reach the maximal fluorescence intensity/h	Fluorescence remaining time/h
Epithelial cells of villi	66.6	3	8
Cancer cells	74.0	6	48

As is shown in table 1, the fluorescence intensity reached the maximal value in just 3 hours in epithelial cells of villi, but 6 hours in cancer cells. The time for the maximal integration of pheophytin in cancer cells is twice as long and fluorescence duration is 6 times as those in epithelial cells of villi, respectively.

Table 2. Characteristics of integration of pheophorbide in cancer cells and normal cells

Type of cells	Maximal fluorescence intensity	Time to reach the maximal fluorescence intensity/h	Fluorescence remaining time/h
epithelial cells of villi	71.0	3	8
Cancer cells	96.3	6	48

As is shown in table 2, the same results were obtained for pheophorbide, but the change of fluorescence intensities between epithelial cells of villi and cancer cells was greater than the case of pheophytin.

Conclusion

Pheophytin and pheophorbide are integrated maximally within 6 hours into cancer cells and the fluorescence is sustained for 48 hours.

The time for pheophytin and pheophorbide to be integrated maximally in cancer cells is twice and fluorescence sustaining time is 6 times as much as those in epithelial cells of villi, respectively.

References

[1] 김일성종합대학학보(자연과학), 46, 9, 116, 주체89(2000).

[2] 김일성종합대학학보(자연과학), 44, 8, 74, 주체87(1998).

[3] S. B. Brown et al; Oncology, 5, 479, 2004.

[4] 刘全宏 等; 动物学扳, 5, 6, 1073, 2005.