Mechanism for Localization of Pheophorbides in Tumor. I. Biodistribution and Subcellular Distribution of 14C-Labeled Pheophorbide and Chlorin in Tumor-Bearing Mice

III. 10. Mechanism for Localization of Pheophorbides in Tumor. I. Biodistribution and Subcellular Distribution of ^{14}C-Labeled Pheophorbide and Chlorin in Tumor-Bearing Mice

*Iwai K., Kimura S. and Ido T.**

Department of Food Chemistry, Tohoku University
*Cyclotron and Radioisotope Center, Tohoku University**

Introduction

Since Dougherty et al[1] reported the therapeutic efficiency of hematoporphyrin deivative (HPD) and light in tumor treatments, this modality has been applied for cancer phototherapy in various organs.[2),3)] The mechanism of photodynamic inactivation on tumor cell has been thought the oxidation of substrates in tumor cell by excited singlet oxygen ($^1O_2$), which HPD produced with irradiation.[4)]

Pheophorbide a (Pheo; Fig.1), a decomposition product from chlorophyll a without magnesium and phytyl group, has been calrified to cause photosensitivities in human and animals.[5)] Kimura et al[6)] proved that Pheo produced $^1O_2$ with light as well as HPD. We have reported the therapeutic efficiencies of Pheo and chlorine e_6Na (Chl;Fig.1), a new derivative of Pheo, in the phototherapy of experimental tumors.[7),8)] On the other hand, vanadium-48-labeled Pheo and Chl chelates have been synthesized to demonstrate their tumor-imaging efficiencies using the affinities of Pheo and Chl with tumor tissues.[9),10)] porphyrin compounds are well known to have affinities with malignant tissues, but there are few report of the localizing mechanism. For the purpose of elucidation of localizing mechanism and the components in tumor cell, which was caused damages by $^1O_2$, we prepared ^{14}C-labeled Pheo and Chl ([^{14}C]pheo and [^{14}C]Chl), and determined the biodistribution and subcellular distribution in tumor-bearing mice.

Materials and Methods

Animal and tumor. Male C3H/He mice were used. FM3A cell, originated from a mammary carcinoma, was implanted subcutaneously into the back of mice.

Preparation of [^{14}C]Pheo and [^{14}C]Chl. ^{14}C-labeled green pigments extract from 93 MBq of [^{14}C]Algae (ICN Radiochemical Co.) and an acetone extract of green pigments from

spinach were mixed. [^{14}C]Chlorophyll a was extracted from ^{14}C-labeled green pigments according to the common method.[11]

[^{14}C]Pheo and [^{14}C]Chl were prepared from [^{14}C]chlorophyll a by our method.[9),10)] The purities were checked by thin layer chromatography. The specific activities of [^{14}C]Pheo and [^{14}C]Chl were 1.40 and 1.24 MBq/mg, radiochemical yields from [^{14}C]Algae were 21 and 18 %, respectively.

Biodistribution. [^{14}C]Pheo was emulsified in phospholipid solution, and [^{14}C]Chl was dissolved in PBS. The mice bearing FM3A tumors were received intravenously with 14.8 kBq of [^{14}C]Pheo and [^{14}C]Chl. At 2, 6, 12 and 24 h after injection, animals were sacrificed by cervical dislocation. Tumor and organs were excised after collection of blood and perfusion of liver with 0.9 % NaCl solution. All organs were resolved in 1.0 ml of Soluene-350 (Packard Co.) with heat. The solution was mixed with 10 ml of Hionic fluor (Packard Co.), then the radioactivity of ^{14}C was measured by a liquid scintillation counter. The concentration was expressed as the percentage of injection dose per gram of tissue (5 dose/g tissue).

Subcellular distribution. From tumor tissue, plasma membrane fraction (Mem.F.) and nuclear fraction (Nuc.F.) were isolated according to the method of Shimizu.[12)] Then mitochondrial fraction (Mit.F.), microsomal fraction (Mic.F.) and cytosol fraction (Cyt.F.) were separated from the residual supernatant by Hogeboom's method.[13)] After isolation of Mem.F. and Nuc.F. from liver and kidney by the method of Yamamoto et al.[14)], Mit.F., Mic.F. and Cyt.F. were separated according to the method of Ouchi et al.[15)] After resolution of fraction in 0.5 ml of Soluene-350, they were mixed with 10 ml of Hionic fluor, then ^{14}C in fraction was measured by a liquid scintillation counter. When radioactivity in Mem.F., Nuc.F., Mit.F., Mic.F., and Cyt.F., are expressed as A (dpm), B(dpm), C(dpm), D(dpm) and E(dpm), respectively, distribution in the Mem.F. can be calculated by the following formula;

$$A/(A+B+C+D+E) \times 100\ (\%).$$

Distribution in the Nuc.F., Mit.F., Mic.F., and Cyt.F. were calculated by substitution of A with B, C, D, E, respectively, in the numerator.

Results and Discussion

Biodistribution of [^{14}C]Pheo and [^{14}C]Chl in mice bearing FM3A tumors are shown in Fig.2 and Fig.3. [^{14}C]Pheo localized into the tumor tissue at 24 h after injection, whereas the high concentration of [^{14}C]Chl in tumor tissue was observed between 6 and 12 h following administration. There was no difference between maximal localization of [^{14}C]Pheo and [^{14}C]Chl. Plasma uptake of both compounds was same at 2 h, but that of

[^{14}C]Chl was lower than that of [^{14}C]Pheo at 24 h. Since all organ levels of [^{14}C]Pheo were higher than those of [^{14}C]Chl except for the kidney and urine levels, [^{14}C]Chl was suggested to be rapidly excreted from the body. High uptake of [^{14}C]Pheo in the spleen and red blood cell is agreeable to the reports that Pheo caused hemolysis by irradiation.[6,16] The Difference between biodistribution of [^{14}C]Pheo and [^{14}C]Chl suggests the difference of their metabolisms, which [^{14}C]Pheo was mainly catabolized in liver and excreted into feces; [^{14}C]Chl was excreted into urine.

Table 1 presents the subcellular distribution of [^{14}C]Pheo and [^{14}C]Chl in FM3A tumor tissue. [^{14}C]Pheo and [^{14}C]Chl mainly distributed in the Mem.F., there was no difference between the both uptakes. The both distribution in the Mit.F. increased with the time, the Cyt.F. uptake decreased. In the Nuc.F. uptake of [^{14}C]Pheo increased with the time, and was higher than that of [^{14}C]Chl at 12 and 24 h after administration.

The subcellular distribution of [^{14}C]Pheo and [^{14}C]Chl in the liver and kidney are presented in Table 2 and Table 3. [^{14}C]Pheo and [^{14}C]Chl also distributed in the Mem.F. of liver. In the kidney, [^{14}C]Pheo was taken in the Mem. F., but the distribution of [^{14}C]Chl in the Cyt.F. were higher than those in the Mem.F. The Nuc.F. uptake of [^{14}C]Pheo were higher than that of [^{14}C]Chl in both organs, and increased with the time. The accumulation of [^{14}C]Pheo in the Nuc.F. was found to be related to the mutagenicity of Pheo.[17] Pheo has a mutagenicity under light irradiation, but has a inhibitory effect of the mutagenicity of benzo(a)pyrene without irradiation. In the Mic.F., higher uptake of [^{14}C]Chl than that of [^{14}C]Pheo suggests that Chl due to be catabolized by microsomal enzymes more than Pheo, and Chl is rapidly excreted from the organs. The distribution of [^{14}C]Pheo and [^{14}C]Chl in the Cyt.F. decreased with the time, and the former was lower than the latter in all organs. High distribution of [^{14}C]Chl in the Cyt.F.was suggested to be induced by its hidrophilic property.

The subcellular distribution of [^{14}C]Pheo and [^{14}C]Chl in the Mem.F. suggests the photodynamic actions of Pheo and Chl with irradiation on cell membrane through the oxidation of lipids and proteins by $^1O_2$.[6] On the other hand, Pheo and Chl were found to bind strongly with the plasma membrane in the tumor more than in the liver and kidney. The strong binding of Pheo and Chl suggests to be reflected on the affinity with the tumors tissues. Latter excretion of Pheo and Chl from tumor tissue than from normal tissues is suggested to be one of the causes of localization into the tumor tissue, because Pheo and Chl bind strongly with the plasma membrane of tumor, and cannot selectively localize into the tumor tissue.

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Table 1 Subcellular distribution of [^{14}C]Pheo and [^{14}C]Chl in FM3A tumor of tumor-bearing mice at various times after injection

Fraction	Distribution (%)			
	2 h	6 h	12 h	24 h
		[^{14}C]Pheo		
Mem.F.	41.2±4.07	39.0±3.22	38.2±3.23	36.8±2.64
Nuc.F.	14.8±1.40	15.7±1.47	17.2±1.86[a]	20.6±1.41[b]
Mit.F.	14.3±0.49	16.5±1.46	18.9±2.19	17.6±1.13
Mic.F.	13.1±1.73	11.2±1.35	10.4±1.58	11.7±0.73
Cyt.F.	16.5±0.95	17.6±1.68	15.3±1.23	13.2±1.37
		[^{14}C]Chl		
Mem.F.	39.3±3.19	38.5±1.65	40.3±4.84	38.9±3.56
Nuc.F.	12.3±1.60	13.7±0.73	11.8±0.55[a]	10.8±1.50[b]
Mit.F.	15.1±1.37	17.1±2.68	18.1±2.40	20.8±1.89
Mic.F.	10.3±0.60	10.2±1.34	14.8±1.54	15.4±1.53
Cyt.F.	23.1±2.99	20.6±1.77	14.9±1.41	14.1±1.94

Mem.F., Plasma membrane fraction; Nuc.F., nuclear fraction; Mit.F., mitochondrial fraction; Mic.F., microsomal fraction; Cyt.F., cytosol fraction. Values are means±SE of four mice. Significant difference between [^{14}C]Chl and [^{14}C]Pheo, [a]$p<0.05$; [b]$p<0.01$.

Table 2 Subcellular distribution of [^{14}C]Pheo and [^{14}C]Chl in liver of tumor-bearing mice at various times after injection

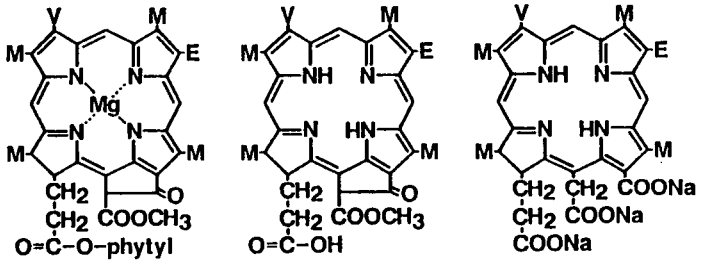
Fraction	Distribution (%)			
	2 h	6 h	12 h	24 h
		[^{14}C]Pheo		
Mem.F.	31.3±3.39	29.8±2.80	31.1±3.62	30.8±2.51
Nuc.F.	17.2±1.72[b]	17.6±2.33[a]	20.7±1.99[b]	24.1±1.56[c]
Mit.F.	18.4±1.35	20.1±2.15	20.0±1.62	20.1±2.17
Mic.F.	12.1±0.41	14.4±1.96	13.8±1.39	12.5±1.15[a]
Cyt.F.	21.0±2.28	18.1±1.40	14.4±2.35	12.3±2.32
		[^{14}C]Chl		
Mem.F.	31.3±2.79	30.0±2.90	31.7±4.66	28.4±3.20
Nuc.F.	9.54±0.85[b]	10.9±0.90[a]	10.1±0.95[b]	11.8±1.50[c]
Mit.F.	17.5±1.35	17.9±1.42	22.9±2.16	23.1±1.56
Mic.F.	12.2±1.54	16.6±1.29	16.0±1.95	18.3±1.29[a]
Cyt.F.	29.5±2.68	24.5±2.89	19.4±1.98	18.5±1.88

Mem.F., Plasma membrane fraction; Nuc.F., nuclear fraction; Mit.F., mitochondrial fraction; Mic.F., microsomal fraction; Cyt.F., cytosol fraction. Values are means±SE of four mice. Significant difference between [^{14}C]Chl and [^{14}C]Pheo, [a]$p<0.05$; [b]$p<0.01$; [c]$p<0.001$.

Table 3 Subcellular distribution of [^{14}C]Pheo and [^{14}C]Chl in kidney of tumor-bearing mice at various times after injection

Fraction	Distribution (%)			
	2 h	6 h	12 h	24 h
		[^{14}C]Pheo		
Mem.F.	31.2±3.02	29.5±2.64	28.4±2.99	28.0±2.02
Nuc.F.	11.5±0.87	14.1±2.06	18.7±1.97[a]	20.4±1.33[b]
Mit.F.	16.8±1.78	18.5±2.39	20.1±1.41	18.2±1.89
Mic.F.	12.8±0.87	11.7±0.96[a]	13.0±1.05	15.2±1.14
Cyt.F.	27.6±2.21[a]	26.2±2.43	19.8±1.81	18.2±1.60[a]
		[^{14}C]Chl		
Mem.F.	24.2±2.60	26.2±3.05	25.3±2.40	24.8±2.47
Nuc.F.	8.53±0.92	11.0±0.82	12.2±1.13[a]	13.2±1.01[b]
Mit.F.	14.8±1.09	16.0±1.90	20.2±2.48	18.1±1.69
Mic.F.	13.2±0.72	16.8±1.64[a]	15.5±0.72	16.9±0.69
Cyt.F.	39.3±3.00[a]	30.0±2.65	26.7±2.23	27.0±2.92[a]

Mem.F., Plasma membrane fraction; Nuc.F., nuclear fraction; Mit.F., mitochondrial fraction; Mic.F., microsomal fraction; Cyt.F., cytosol fraction. Values are means±SE of four mice. Significant difference between [^{14}C]Chl and [^{14}C]Pheo, [a]$p<0.05$; [b]$p<0.01$.



Chlorophyll a Pheophorbide a Chlorin e_6Na

Fig. 1. Chemical structures of chlorophyll a Pheo, and Chl
M, -CH_2; E, -CH_2CH_3; V, -CH=CH_2; phytyl,
-$CH_2CHCH_2(CH_2CH_2CHCH_2)_2CH_2CH_2C$=$CHCH_2OH$.
| | |
CH_3 CH_3 CH_3

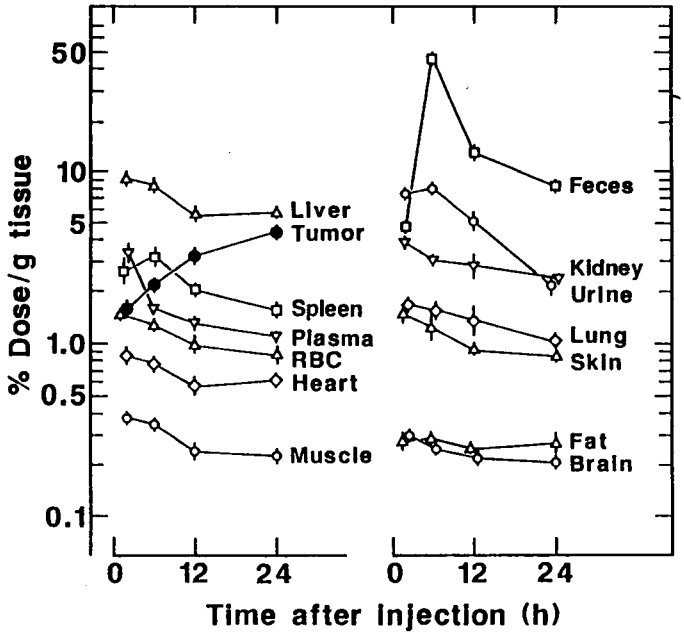


Fig. 2. Biodistribution of [^{14}C]Pheo in C3H mice bearing FM3A tumors
Bars, SE of four mice.

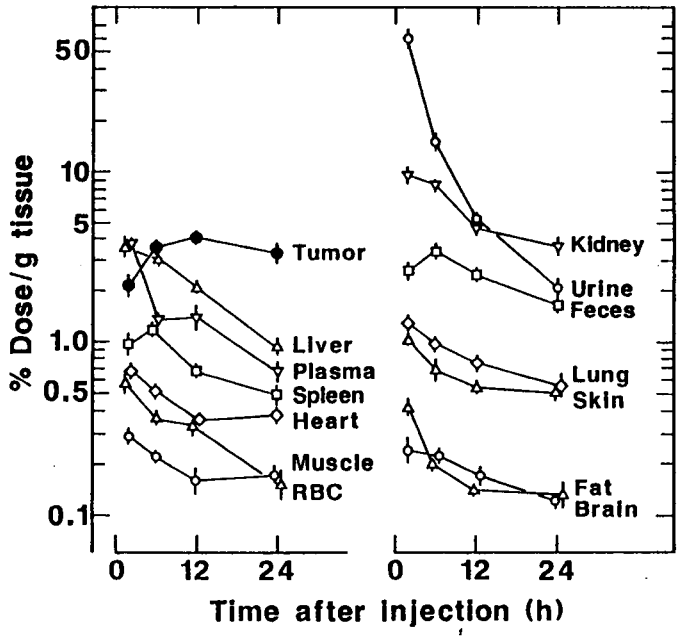


Fig. 3. Biodistribution of [^{14}C]Chl in C3H mice bearing FM3A tumors
Bars, SE of four mice.