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Photodynamic Effect of Polyethylene Glycol-modified Fullerene on Tumor

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Fullerene (C_{60}) efficiently generates singlet oxygen when irradiated with light, and thus should have a photodynamic effect on tumors, if it is accumulated in the tumor tissue. To explore tumor targeting of C_{60}, we chemically modified the water-insoluble C_{60} with polyethylene glycol (PEG), not only to make it soluble in water, but also to enlarge its molecular size. When injected intravenously into mice carrying a tumor mass in the back subcutis, the C_{60}-PEG conjugate exhibited higher accumulation and more prolonged retention in the tumor tissue than in normal tissue. The conjugate was excreted without being accumulated in any specific organ. Following intravenous injection of C_{60}-PEG conjugate or Photofrin® to tumor-bearing mice, coupled with exposure of the tumor site to visible light, the volume increase of the tumor mass was suppressed and the C_{60} conjugate exhibited a stronger suppressive effect than Photofrin. Histological examination revealed that conjugate injection plus light irradiation strongly induced tumor necrosis without any damage to the overlying normal skin. The antitumor effect of the conjugate increased with increasing irradiation power and C_{60} dose, and cures were achieved by treatment with a dose of 424 μg/kg at an irradiation power of 107 J/cm^2. These findings indicate that PEG-modified C_{60} is a candidate agent for photodynamic tumor therapy.

Key words: Fullerene — Polyethylene glycol — Chemical conjugation — Photodynamic effect — Tumor

Various porphyrin derivatives have been explored as potential photosensitizing compounds for photodynamic tumor therapy. It has been well recognized that singlet oxygen generated from these compounds through light irradiation acts as an effective cytotoxic agent.[1] Fullerene (C_{60}) efficiently generates singlet oxygen when exposed to visible light.[2] Thus, if C_{60} can be preferentially accumulated in tumor tissue, irradiation with visible light should induce tumor necrosis as a result of generation of singlet oxygen from the irradiated C_{60}.

Hyperpermeable tumor vasculature and immature lymph systems have been reported to allow large-sized substances to accumulate and show prolonged retention at tumor tissues in preference to normal tissue.[3–11] It has also been demonstrated that passive targeting of antitumor drugs to a tumor site can be achieved by increasing the apparent molecular size through conjugation with polymers.[4, 5]

The objective of this study was to target C_{60} to a tumor tissue by making C_{60} soluble in water and increasing its apparent molecular size through conjugation with polyethylene glycol (PEG), which has been widely used for chemical modification of drugs because of its simple conjugation chemistry.[12–14] Following intravenous injection of water-soluble C_{60}-PEG conjugate into tumor-bearing mice and the subsequent exposure to visible light, the photodynamic effect of C_{60}-PEG conjugate on the tumor was investigated in comparison with that of clinically used "Photofrin." To our knowledge, no application of large-sized, water-soluble C_{60} for photodynamic therapy has been reported, although polymerized C_{60} and C_{60} modification with polymers have been studied for reasons unrelated to anti-tumor effect.[15–17] Here we describe the tumor accumulation of C_{60}-PEG conjugate and its toxicity to mice.

MATERIALS AND METHODS

Materials C_{60} (99.9%, MW=720.66, Lot No. FHC03) was obtained from Tokyo Kasei Kogyo Co., Ltd., Tokyo. Photofrin was purchased from Lederle Ltd., Tokyo and used as its 5% glucose solution. Monomethoxy PEG with a terminal primary amino group was kindly supplied by Nippon Oil & Fats Co., Ltd., Tokyo (PEG, MW=5,460). $Na^{125}I$ (20 mCi/ml, 0.1 *N* NaOH aqueous solution) and an anion-exchange resin, Dowex 1-8X, were purchased from NEN Research Products, DuPont, Wilmington, DE and Dow Chemicals Co., Ltd., Midland, MI, respectively. Other chemicals were used without further purification.

Chemical conjugation of C_{60} with PEG Chemical conjugation of PEG to C_{60} was based on the high coupling reactivity of amines to C_{60}.[18] A given amount of PEG was mixed with C_{60} in benzene solution and the coupling reaction was allowed to proceed under stirring at 25°C for 24 h in the dark, followed by freeze-drying to obtain

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a powdered C_{60}-PEG conjugate. The solubility of PEG-modified C_{60} in water was qualitatively assessed by determining the water/benzene partition ratio.[17] The weight-average molecular weight of the C_{60}-PEG conjugate was estimated by using gel filtration chromatography (GFC, Tosoh Corporation, Tokyo) with standard PEG samples.

Preparation of mice carrying a tumor mass in the back subcutis Meth A fibrosarcoma cells, acclimatized to *in vivo* conditions, were inoculated at a volume of 0.05 ml into the back subcutis of female CDF_1 mice aged 6 weeks (Japan SLC, Inc., Shizuoka) (3×10^6 cells/mouse). When the tumor mass at the inoculation site reached 7 mm in average diameter, the tumor-bearing mice were used for experiments.

Radioiodination of C_{60}-PEG conjugate Radioiodination of C_{60}-PEG conjugate was conducted according to a chloramine T method following tyramine introduction into the conjugates.[19] Briefly, 2.7 mg of tyramine was added to a dimethyl sulfoxide solution containing 100 mg of C_{60}-PEG conjugate at a tyramine/C_{60} molar ratio of 100. After coupling reaction at 25°C for 1 day, the resulting solution was dialyzed against double-distilled water for 2 days using a Spectrapore dialysis membrane with a cutoff molecular weight of 1,000 (Medical Industries, Inc., Los Angeles, CA) to exclude non-coupled tyramine, followed by freeze-drying to obtain tyramine-bearing C_{60}-PEG conjugate.

The conjugate was dissolved in 150 μl of 0.5 *M* potassium phosphate-buffered (KPB) solution (pH 7.5) to give a final concentration of 50 μg/ml. Then, 2 μl of $Na^{125}I$ solution (40 μCi) and 100 μl of 0.05 *M* KPB solution (pH 7.2) containing 0.02 mg of chloramine T were added to the C_{60}-PEG conjugate solution. The mixture was agitated at 25°C for 2 min, then 100 μl of 0.01 *M* phosphate-buffered saline (PBS) solution (pH 7.4) containing 0.4 mg of sodium pyrosulfite was added to stop radioiodination. The resulting mixture was applied to a column of Dowex resin to remove uncoupled, free ^{125}I molecules from the ^{125}I-labeled C_{60}-PEG conjugate.

Body distribution measurement of C_{60}-PEG conjugate following intravenous injection Mice carrying a tumor mass in the back subcutis received intravenous injection of 100 μl of PBS solution containing 0.02 wt% of ^{125}I-labeled C_{60}-PEG conjugate. Each experimental group was composed of 4 mice. At different time intervals, blood samples were taken directly from the heart by syringe aspiration, and organs and the carcass (residual body portions) were separated. The radioactivity of the excised body organs after washing twice with PBS and that of the blood samples was measured with a gamma counter (Autowell gamma system Aloka ARC-301B, Aloka Co., Ltd., Tokyo). The urine and feces of mice were collected and the percentage of radioactivity with respect to that injected was taken as a measure of excretion of the conjugates. The radioactivity of each organ was calculated by subtracting the radioactivity of blood present therein from the total radioactivity.[19]

Mouse skin samples (1.5×1.5 cm^2) with and without the tumor mass were taken from tumor-bearing mice at their back subcutis and the radioactivity was measured to calculate the radioactivity of the tumor tissue itself from their difference.

The body distribution data were expressed as percent radioactivity and % dose/g tissue, which are the percentage of radioactivity measured with respect to that injected per mouse and the quotient divided by the weight of each tissue, respectively.

Assessment of photodynamic effect of C_{60}-PEG conjugate on the tumor PBS solutions containing C_{60}-PEG conjugate with various amounts of C_{60} (0.1 ml/mouse) were intravenously injected into mice carrying a tumor mass in the back subcutis. The injection dose of C_{60} was changed by altering the dose of C_{60}-PEG conjugate. At 24 h later, the tumor site was exposed to visible light (400–505 nm) from the light probe (7 mm active diameter) of a Heliomat Multifunction Halogen-Light (Vivadent Co., Ltd., Lichtenstein). The light was delivered at a fluence of 89.2 mW/cm^2 for various time periods. The tumor size was evaluated according to the method reported by Winn[20] and expressed as the volume ratio of the tumor to that before conjugate injection. As controls, intravenous injection of C_{60}-free PEG with or without the subsequent light irradiation and C_{60}-PEG conjugates alone or light irradiation without any injection was performed. For comparison, 0.1 ml of an aqueous glucose solution containing 80 μg of Photofrin (4 mg/kg) was intravenously injected into the tumor-bearing mice and the tumor site was exposed to visible light (610–800 nm) at 72.5 mW/cm^2, 107 J/cm^2 at 24 h after the injection. Since light with the wavelength of 620 nm is normally used for photodynamic therapy with Photofrin, we used visible light with total irradiation power equivalent to that used for the conjugate. Each experimental group was composed of 6 mice and all the mice were fed on normal food with water *ad libitum* but in the dark during the experiment.

Tumor-bearing mice receiving intravenous injection of C_{60}-PEG conjugate containing 8.48 μg of C_{60} (424 μg/kg) were treated with or without light irradiation 24 h later. At 24 h after light irradiation, the tumor site was removed, fixed with 10 wt% buffered formalin aqueous solution, sectioned, and stained with hematoxylin-eosin to view the histological response of the tumor tissue.

Toxicity evaluation of C_{60}-PEG conjugate *In vivo* toxicity of C_{60}-PEG conjugate was evaluated in terms of the body weight change and blood examination of mice given the injection and light irradiation at the tumor site. The time profile of body weight of tumor-bearing mice given intraperitoneal or intravenous injection of C_{60}-PEG con-

jugate was measured for comparison with that of control mice.

Next, at 24 h after completion of light irradiation following conjugate injection, the blood was sampled to determine the plasma concentration of glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), and blood urea nitrogen (BUN) as measures of hepatotoxicity and nephrotoxicity, respectively.

RESULTS

Characterization of C_{60}-PEG conjugates The color of the mixture of PEG with C_{60} in benzene changed from violet to brown with time after mixing and the extent of the change depended on the molar ratio of PEG added to C_{60} at the coupling reaction. GFC studies revealed that the peak of PEG itself disappeared, a new peak being de-

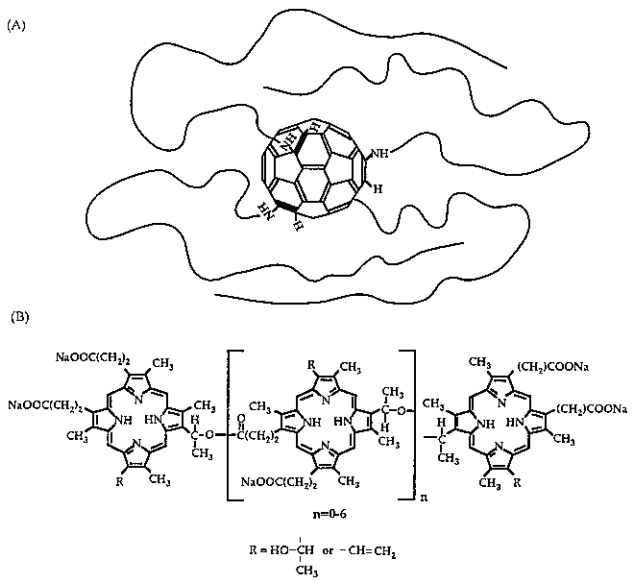

Fig. 1. The chemical structure of C_{60}-PEG conjugate (A) and Photofrin (B).

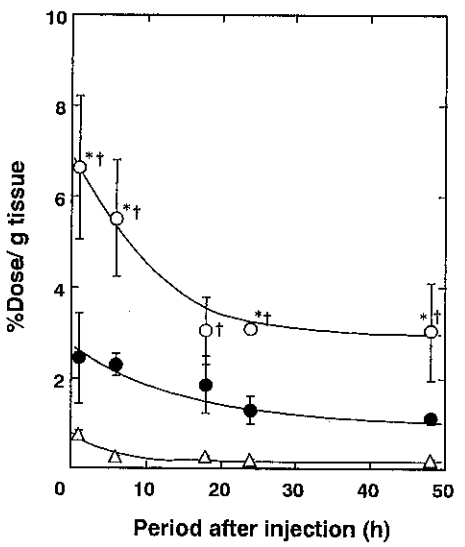

Fig. 2. The time course of C_{60}-PEG accumulation after intravenous injection into tumor-bearing mice. ○, tumor tissue; ●, normal skin; △, normal muscle. * $P<0.05$, significantly different from normal skin. † $P<0.01$, significantly different from normal muscle.

Table I. Time Course of Organ Distribution of the C_{60}-PEG Conjugate[a] after Intravenous Injection into Tumor-bearing Mice

Organ	Percent radioactivity				
	Period after injection				
	1 h	6 h	24 h	72 h	96 h
Blood	16.5±1.50[b]	7.15±1.05	1.89±0.14	0.83±0.07	0.39±0.07
Heart	0.10±0.04	0.07±0.01	0.05±0.01	0.04±0.01	0.04±0.01
Lung	0.18±0.03	0.15±0.05	0.08±0.01	0.06±0.01	0.05±0.01
Liver	3.32±0.88	5.23±0.34	6.18±0.13	2.13±0.11	1.97±0.07
Spleen	0.19±0.05	0.14±0.03	0.18±0.01	0.06±0.01	0.05±0.01
Kidney	2.53±0.18	2.81±0.29	2.17±0.09	1.28±0.12	1.02±0.08
Gastrointestinal tract	5.65±1.75	5.15±0.90	3.03±0.89	5.23±0.07	0.95±0.08
Carcass	33.6±11.5	12.0±0.79	5.67±0.60	5.24±0.37	3.22±0.68
Excreted	33.6±7.80	64.5±0.55	77.6±1.30	79.04±0.57	88.29±0.76

a) The molar ratio of PEG to C_{60} in the coupling reaction was 100.
b) Mean±SE.

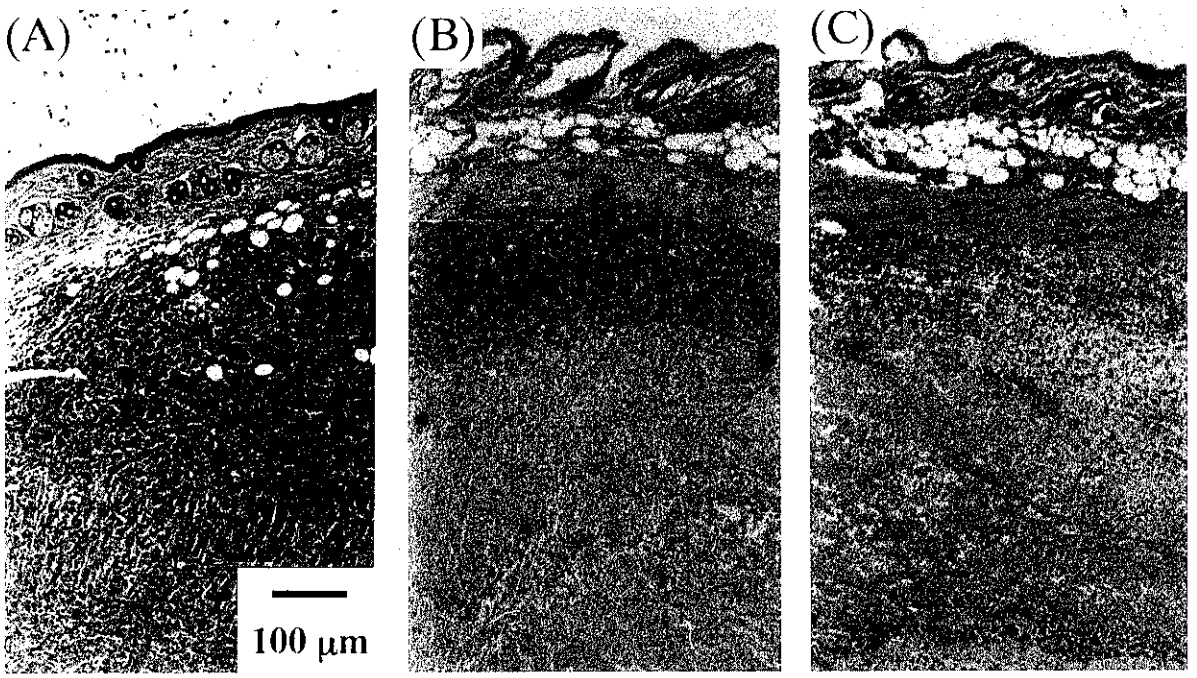

Fig. 3. Histological sections of the tumor site of mice given intravenous injection of the C_{60}-PEG conjugate at a C_{60} dose of 424 μg/kg, followed by light irradiation at 24 h after injection. The irradiation power was (A) 0, (B) 53.5, or (C) 107 J/cm^2.

Table II. Time Course of Tumor Growth in Tumor-bearing Mice Given Intravenous Injection of C_{60}-PEG Conjugate[a] and C_{60}-free PEG, Followed by Light Irradiation

Injection	Light irradiation (J/cm^2)	Tumor volume ratio[b]			
		2 days	6 days	8 days	11 days
C_{60}-PEG conjugate	107	1.10±0.18	0.79±0.24*	0.56±0.14*	0.54±0.05*
C_{60}-PEG conjugate	0	2.00±0.42	3.80±0.72	5.90±1.35	9.75±1.95
C_{60}-free PEG	107	1.35±0.30	3.41±0.95	8.40±2.42	9.89±2.65
C_{60}-free PEG	0	1.84±0.42	3.70±1.20	9.09±2.01	9.87±1.78
PBS	107	1.58±0.21	3.43±1.21	8.51±2.07	10.2±2.23
PBS	0	1.64±0.30	3.51±0.90	8.20±1.20	9.36±1.43

a) The molar ratio of PEG to C_{60} in the coupling reaction was 100.
b) The ratio of the tumor volume at the given days to that at day 0.
* $P<0.05$, significantly different from control mice without light irradiation.

tected at a shorter retention time after C_{60} conjugation. This clearly indicated that chemical coupling of PEG to C_{60} had taken place. When estimated in terms of water/benzene partition, the solubility of C_{60}-PEG conjugate in the water phase increased with increasing PEG/C_{60} molar ratio in the coupling reaction and the conjugate prepared was completely partitioned to the water phase at the ratio of 100. The molecular weight of this completely water-soluble C_{60}-PEG conjugate was approximately 26,000. As the molecular weight of PEG is 5,460, it appears that four PEG molecules were covalently bound to one molecule of C_{60} on average. This completely water-soluble C_{60}-PEG conjugate was used without purification. Fig. 1 shows the chemical structures of the C_{60}-PEG conjugate and Photofrin. The injection dose of C_{60} was calculated from the amount of C_{60} added initially on the assumption that no C_{60} was lost before or after the coupling reaction.

Body distribution of C_{60}-PEG conjugate after intravenous injection Table I shows the distribution of C_{60}-PEG conjugate after intravenous injection into tumor-bearing

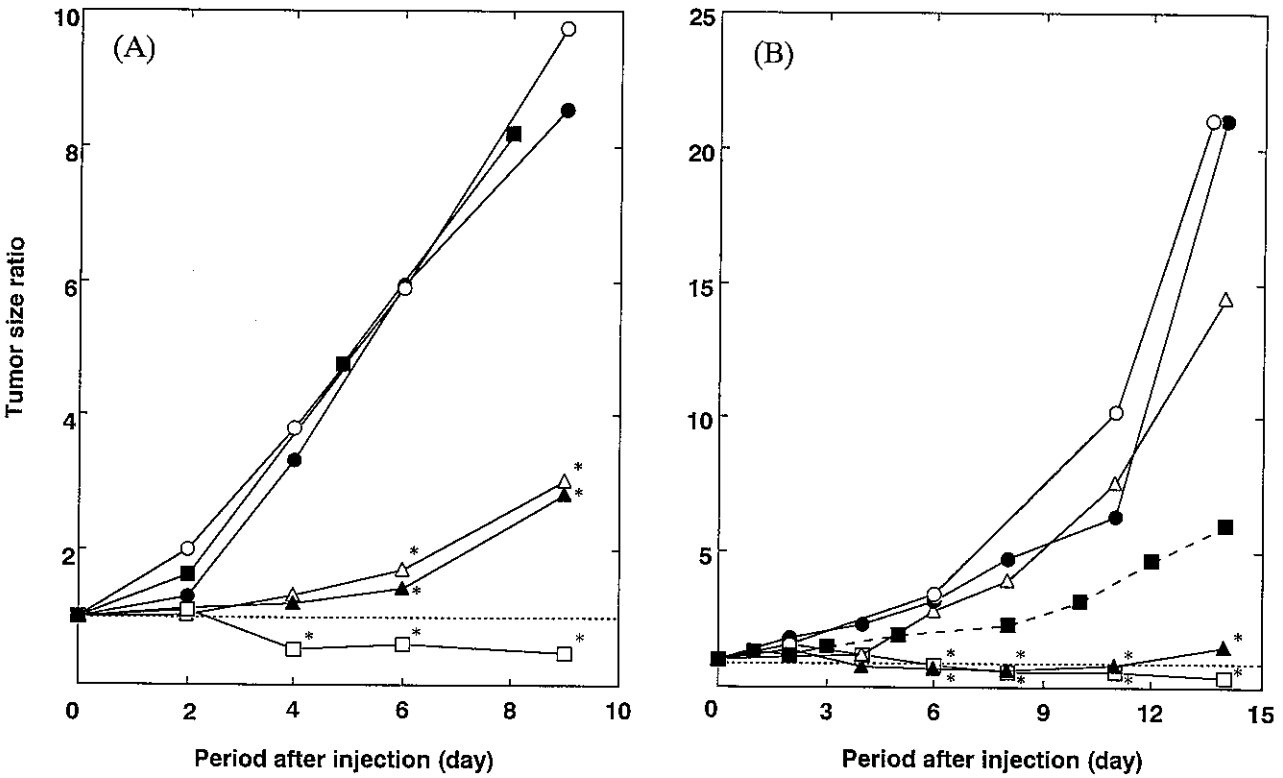

Fig. 4. Photodynamic effect of the C_{60}-PEG conjugate and Photofrin on *in vivo* growth of Meth A fibrosarcoma cells inoculated into the back subcutis of mice. A, Tumor-bearing mice given an intravenous injection of 6 mg of C_{60}-PEG conjugate at a C_{60} dose of 424 μg/kg. The tumor site was irradiated with light at 24 h after injection at a power of (○) 0, (●) 10.7, (△) 26.7, (▲) 53.5, or (□) 107 J/cm^2. (■) indicates PBS-injected, control mice without light irradiation. B, Tumor-bearing mice given an intravenous injection of PBS (○) or C_{60}-PEG conjugate at a C_{60} dose of 42.5 (●), 85.0 (△), 212 (▲), or 424 μg/kg (□), or Photofrin (4 mg/kg) (■), followed by light irradiation at 24 h after injection (400–505 nm, 89.2 mW/cm^2, 107 J/cm^2 for the C_{60}-PEG conjugate and 610–800 nm, 72.5 mW/cm^2, 107 J/cm^2 for Photofrin). $*P<0.05$, significantly different from untreated, control mice. A dotted line indicates the tumor size ratio of 1.0.

mice. The injected C_{60}-PEG conjugate disappeared gradually from the blood circulation and 78% of the injected conjugate was excreted from the body within 24 h. The conjugate did not exhibit marked accumulation in any organ. The liver accumulation tended to increase up to 24 h after injection, but thereafter decreased with time, becoming undetectable at 144 h after injection (data not shown). The conjugate accumulated in the gastrointestinal tract and carcass at an early period after injection, but was eliminated thereafter in the same manner as in the liver.

Fig. 2 shows the time course of accumulation of the C_{60}-PEG conjugate in the tumor and normal skin and muscle. The C_{60}-PEG conjugate was accumulated in the tumor tissue to a significantly higher extent than in the skin and muscle. The conjugate was retained in the tumor tissue in a significantly larger amount for a longer time than in the normal tissues.

Photodynamic effect of C_{60}-PEG conjugate on tumor Fig. 3 shows histological sections of the tumor site of mice receiving intravenous injection of the C_{60}-PEG conjugate followed by light irradiation. Treatment with conjugate injection coupled with light irradiation, induced marked tumor necrosis, whereas the overlying normal skin was not damaged. On the other hand, conjugate injection alone did not induce any tissue necrosis. This finding demonstrates that light irradiation is essential to induce destruction of the tumor tissue of mice given the conjugate injection. When the back skin of the conjugate-injected normal mice was exposed to light at 89.2 mW/cm^2 for 20 min, amounting to 107 J/cm^2, at 24 h after injection, no histological damage to the skin was observed (data not shown).

Table II summarizes the time course of tumor volume of the tumor-bearing mice given intravenous injection of C_{60}-PEG conjugate and other agents. The *in vivo* tumor

growth was significantly suppressed only when conjugate injection was followed by light irradiation. No suppressive effect was observed when either of the two processes was omitted. The time profile of tumor growth of mice treated with C_{60}-free PEG was similar to that of PBS-injected, control mice, irrespective of light irradiation. This indicates that C_{60} itself is the key to achieve light-induced tumor suppression.

Fig. 4 shows the photodynamic effect of C_{60}-PEG conjugate and Photofrin on the *in vivo* growth of Meth A fibrosarcoma cells. The photodynamic effect of C_{60}-PEG conjugate on the tumor greatly depended on the C_{60} dose and the light irradiation power. The *in vivo* suppression of tumor growth by the conjugate increased with increase in the irradiation power (Fig. 4A). When the irradiation power was 107 J/cm^2, the tumor size ratio became less than 1.0 at 4 days after the injection and observation of all the mice for a longer time period revealed disappearance of the tumor mass. The time profile of tumor growth for mice receiving conjugate injection without light irradiation was similar to that for PBS-injected, control mice without light irradiation. This indicates that the conjugate itself did not have any cytocidal effect on the tumor tissue unless light irradiation was performed. An increase in the C_{60} dose of C_{60}-PEG conjugate enhanced the photodynamic effect on the tumor (Fig. 4B). The size increase of the tumor mass was significantly suppressed by conjugate injection followed by light irradiation at a C_{60} dose of more than 212 μg/kg. The treatment with the conjugate at the C_{60} dose of 424 μg/kg decreased the tumor size ratio to less than 1.0 and finally, all the tumor-bearing mice were cured. The photodynamic effect of Photofrin on the tumor was less than that of the conjugate even at 10-times or more higher doses than that of the conjugate. Light irradiation alone did not affect the tumor growth at all.

Toxicity of C_{60}-PEG conjugate Fig. 5 shows the time course of the body weight gain of tumor-bearing mice intraperitoneally injected with the C_{60}-PEG conjugate, followed by light irradiation. There was no significant difference in body weight change between the conjugate-injected and the uninjected mice, irrespective of the C_{60} injection dose, although a temporary but significant weight loss was noticed during the initial 2 days at the highest C_{60} dose. However, the body weight completely recovered to that of other mice and no death was observed. In addition, the time profile of body weight gain of the tumor-bearing mice injected intravenously with the C_{60}-PEG conjugate containing 36 and 360 μg of C_{60} (1.8 and 18 mg/kg) was not significantly different from

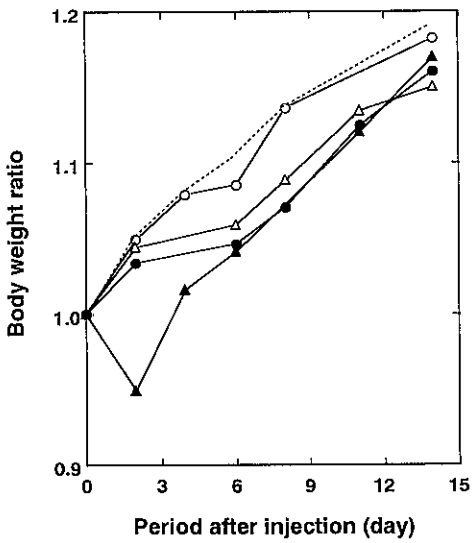

Fig. 5. The time course of body weight change of the tumor-bearing mice after intraperitoneal injection of the C_{60}-PEG conjugate at a C_{60} dose of (○) 1.80, (●) 18.0, (△) 180, or (▲) 1,800 mg/kg, followed by light irradiation to the tumor site at 24 h after injection (107 J/cm^2). A dotted line indicates the weight change of tumor-bearing mice irradiated with light.

Table III. Plasma GOT, GPT, and BUN of Tumor-bearing Mice Given Intravenous Injection of the C_{60}-PEG Conjugate,[a] Followed by Light Irradiation

Mice were injected with	Light irradiation (J/cm^2)	Plasma concentration		
		GOT (IU/liter)	GPT (IU/liter)	BUN (mg/dl)
PBS	107	35±5	30±6	18.6±4.7
PBS	0	29±10	35±4	17.0±6.2
C_{60}-PEG conjugate	107	35±4	28+8	20.6±2.5
C_{60}-PEG conjugate	0	34±6	27±9	21.8±4.0
PBS (normal)[b]	0	32±6	25±8	20.1±3.6
C_{60}-PEG conjugate (normal)[b]	0	32±7	28±5	21.8±3.2

a) The molar ratio of PEG to C_{60} in the coupling reaction was 100.
b) Normal mice were used.

that of light-irradiated, but uninjected, tumor-bearing mice (data not shown). Table III summarizes the blood test results of tumor-bearing mice given intravenous injection of the C_{60}-PEG conjugate and PBS with or without light irradiation. As controls, normal mice were injected with PBS and the conjugate. Plasma concentrations of GOT, GPT, and BUN were not changed by injection of the conjugate and PBS, irrespective of light irradiation, being similar to those of the normal mice injected with PBS. Conjugate injection alone did not affect the plasma concentration of normal mice.

DISCUSSION

Photofrin is a mixture of hematoporphyrin oligomers and is known to have an inherent affinity for low density lipoprotein (LDL), with which it is internalized into tumor cells to a higher extent than into normal cells,[21] because of the higher activity of the LDL receptor of the tumor cells. This feature results in preferential accumulation in the tumor following intravenous injection. In contrast, C_{60} does not have such an affinity for the tumor. Its water-insolubility has prevented C_{60} from being utilized as a photosensitizer, in spite of its photochemical ability to generate singlet oxygen. Many studies have been reported on water solubilization of C_{60}[15–17, 22–27] and some water-solubilized C_{60} derivatives were demonstrated to exhibit oxygen-induced cytotoxic effects *in vitro*.[17, 24–26] A body distribution study revealed that a water-miscible C_{60} derivative injected intravenously was accumulated in the liver at a high level.[27] This result implies that the body distribution of C_{60} must be altered so as to achieve accumulation in the tumor tissue.

As expected, solubilization of C_{60} in water and enlargement of its molecular size through PEG conjugation resulted in higher tumor accumulation of C_{60} and its retention in the tumor tissue for a longer time than in the normal tissue (Fig. 2). We have demonstrated that the molecular weight (26,000) of the present C_{60}-PEG conjugate was high enough to allow preferential accumulation in tumor tissues based on their anatomical features.[28] The ratio of conjugate accumulation in the tumor tissue to that in the normal skin and muscle was 2.7 and 19, respectively, at 24 h after intravenous injection. This clearly indicates that tumor targeting of C_{60} was realized, suggesting that light irradiation at the tumor site would result in a high photodynamic effect on the tumor.

Table I reveals no specific affinity of C_{60}-PEG conjugate for any organ. This may be ascribed to the nature of PEG. Because the surface of the C_{60}-PEG conjugate is covered with PEG molecules, it would be difficult for the conjugate to interact with body components, such as proteins, lipids, LDL, and cells. It is likely that the intravenously injected conjugate is finally excreted via the kidney, because the molecular weight of 26,000 is low enough for glomerular filtration to occur.[19] Since the liver vasculature is composed of discontinuous vascular walls, unlike other organs, the C_{60}-PEG conjugate may be distributed in the extravascular tissue after intravenous injection, but may return to the blood circulation by diffusion because the conjugate concentration in the blood is always low due to quick elimination from the bloodstream. As a result, the C_{60}-PEG conjugate will be accumulated in the liver during the first day after injection.

We have already reported that PEG-modified C_{60} suppresses *in vitro* growth of normal cells when exposed to visible light, but does not exhibit any suppressive effect on cell growth in the absence of light irradiation.[17] Interestingly, the present histological examination revealed that light irradiation at the tumor site strongly induced tumor necrosis without any damage to the overlying normal skin, although the light penetrated the skin to reach the tumor tissue. The C_{60}-PEG conjugate preferentially accumulated in the tumor tissue must be photodynamically activated by light irradiation to generate singlet oxygen, which will cause tumor necrosis without damage to the normal tissue.

The C_{60}-PEG conjugate exhibited a stronger photodynamic effect than Photofrin although the C_{60} dose was 10-times or more less than that of Photofrin. When the absorption spectra of both compounds were compared, the C_{60} conjugate has a small absorptivity in the wavelength region where light has optimal penetration through tissue, i.e., in the 600–900 nm region,[29] compared with Photofrin. However, this effect is not sufficient to explain the difference in the photodynamic efficiency of the two compounds. In addition, when light of 400–505 nm was used on tumor-bearing mice injected with Photofrin, the photodynamic effect on the tumor was similar to that in Fig. 4 (data not shown). The photodynamic effect would also be influenced by other factors.

Hematoporphyrin derivatives and Photofrin are retained in the skin for at least 4–6 weeks, thereby causing severe skin sensitization to sunlight.[30] This might be ascribed to an inherent affinity of the hematoporphyrin for cells via the LDL receptors. However, C_{60}-PEG conjugate does not have such a biospecific interaction with cells. Indeed, the conjugate was excreted within 1 week through the kidneys without long-term retention in normal tissues, which presumably results in less skin sensitization.

The highest injection dose of C_{60} (1.8 g C_{60}/kg), at which no mouse death was observed, was approximately 4,000 times that required for a significant photodynamic effect of the conjugate. It was reported that the LD_{50} value of Photofrin following intraperitoneal injection to normal mice was 130 mg/kg (Lederle Japan Ltd., un-

published data). The additional light irradiation may result in a reduced LD_{50} value, depending on the irradiation protocol. Nevertheless, a simple comparison suggests lower toxicity of our water-soluble C_{60}-PEG conjugate than that of Photofrin.

In conclusion, PEG conjugation with C_{60} not only makes C_{60} soluble in water, but also increases the molecular size so as to allow preferential accumulation of C_{60} in tumor tissue. These features resulted in passive tumor targeting by C_{60}-PEG conjugate, allowing selective destruction of tumor tissues through subsequent light irradiation at the tumor site. This finding demonstrates that the C_{60}-PEG conjugate is a promising candidate for photodynamic tumor therapy.

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