Pharmaceutical Nanotechnology

Evaluation of Photodynamic Activity of C_{60}/2-Hydroxypropyl-β-Cyclodextrin Nanoparticles

DAISUKE IOHARA,[1] MASAAKI HIRATSUKA,[2] FUMITOSHI HIRAYAMA,[1] KEIZO TAKESHITA,[1] KEIICHI MOTOYAMA,[2] HIDETOSHI ARIMA,[2] KANETO UEKAMA[1]

[1]Faculty of Pharmaceutical Sciences, Sojo University, Kumamoto 860-0082, Japan

[2]Graduate School of Pharmaceutical Sciences, Kumamoto University, Kumamoto 862-0973, Japan

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ABSTRACT: The objective of this study is to evaluate the ability of C_{60}/2-hydroxypropyl-β-cyclodextrin (HP-β-CyD) naonparticles to generate reactive oxygen species (ROS) and to induce cell toxicity by the photoirradiation. C_{60} nanoparticles were prepared by cogrinding with HP-β-CyD for 3 h at 4°C under reduced pressure. The photodynamic activity of C_{60}/HP-β-CyD nanoparticles was evaluated by spectroscopic methods, including the electron spin resonance spin-trapping method, and by the cell viability test using Hela cells. C_{60}/HP-β-CyD nanoparticles efficiently generated not only superoxide anion radical ($O_2^{\cdot-}$) and hydroxyl radical (·OH), but also singlet oxygen ($^1O_2$) through photoirradiation. The ROS generation was enhanced by decreasing the mean particle diameter of C_{60} nanoparticles, and the particle size smaller than 90 nm showed a high generation of ·OH and $^1O_2$. In addition, HP-β-CyD enhanced the generation of $^1O_2$, compared with polyvinylpyrrolidone (an effective solubillizer for C_{60}), due to partial disposition of C_{60} in the hydrophobic CyD cavity. Furthermore, C_{60}/HP-β-CyD nanoparticles showed cell toxicity after the light irradiation, but no toxicity was observed without the light irradiation. Therefore, HP-β-CyD is useful for the preparation of stable C_{60} nanoparticles with high ROS generation ability, and C_{60}/HP-β-CyD nanoparticles are a promising photosensitizer for photodynamic therapy. © 2012 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 101:3390–3397, 2012

Keywords: C_{60}; 2-Hydroxypropyl-β-cyclodextrin; Nanoparticles; Electron spin resonance (ESR); Reactive oxygen species; Photodynamic therapy

INTRODUCTION

Photodynamic therapy (PDT) is a next-generation cancer treatment based on reactive oxygen species (ROS). In PDT, a photosensitizer is systemically, locally, or topically administered, and tumor sites are irradiated by visible lights to generate ROS site-selectively, leading to cell deaths and tissue destructions.[1–3] Therefore, PDT is an effective method for destroying diseased tissues without damaging the surrounding healthy tissues. The ideal photosensitizer for PDT is required to have high quantum yields in the generation of ROS, effective absorption of long-wavelength lights, and low toxicity under nonphotoirradiation. Many clinically employed photosensitizers are based on porphyrin molecules such as a hematoporphyrin derivative, Photofrin®. However, these compounds have several disadvantages such as prolonged skin sensitivity necessitating avoidance of sunlight for few weeks,[4] suboptimal tumor selectivity,[5] and poor light penetration into tumors[6] due to the absorption of the relatively short wavelengths (630 or 664 nm). Therefore, more efficient photosensitizers are in various stages of development to improve their usefulness.[7]

Fullerenes are currently of great interest for practical applications that take advantage of their unique electronic properties and biological activities.[8] The fullerene family, especially C_{60}, has appealing photochemical, electrochemical, and physical properties, which can be exploited in various medical fields.[9] Specifically, C_{60} has been regarded as an efficient photosensitizer for PDT[10,11] due to the light absorption of relatively long wavelengths (S–S absorption: 530 and

Correspondence to: Fumitoshi Hirayama (Telephone: +81-96-326-4096; Fax: +81-96-326-4096; E-mail: fhira@ph.sojo-u.ac.jp), Kaneto Uekama (Telephone +81-96-326-4096; Fax: +81-96-326-4096; E-mail:uekama@ph.sojo-u.ac.jp)

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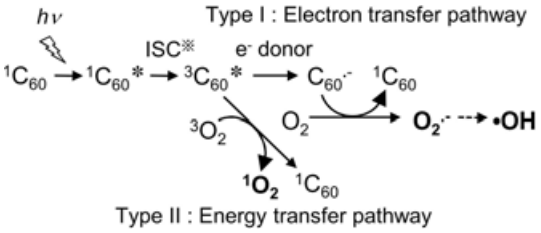

Figure 1. ROS generation pathway by C_{60} with photoirradiation. ※Intersystem crossing.

620 nm; T–T absorption: 400 and 740 nm) and the high quantum yield of photoexcitation reactions.[12,13] Photoirradiation of C_{60} results in the formation of the singlet excited state, $^1C_{60}$*, followed by conversion to a triplet state, $^3C_{60}$*, through the intersystem crossing with the high quantum yield (Fig. 1). Because the reduction potential of $^3C_{60}$* is significantly high,[14] an electron transfer from reductants such as amines and NADH to $^3C_{60}$* occurs to give the $C_{60}^{\cdot-}$ radical anion, subsequently reducing oxygen to superoxide anion radial ($O_2^{\cdot-}$) (type I pathway).[15,16] In addition, the energy transfer reactions (type II pathway) of photoexcited C_{60} have been reported,[12] that is, the $^3C_{60}$* efficiently transfers its energy to molecular oxygen to give singlet oxygen ($^1O_2$). In spite of these potential photoinduced biological activities of C_{60}, its extremely low solubility and poor dispersibility in water have significantly impeded pharmaceutical applications.[17]

Although several water-soluble fullerene derivatives are reported,[18–23] chemical modifications of C_{60} usually decrease its photophysical properties.[24,25] Therefore, solubilization of C_{60} without chemical modifications is a better approach for pharmaceutical applications of C_{60}. Furthermore, the large aggregation of C_{60} significantly accelerates the decay of excited triplet state C_{60}, thus, reducing the photosensitizing ability of C_{60}.[26] In a previous study, we reported the formation of stable C_{60} nanoparticles, on whose surfaces hydrophilic 2-hydroxypropyl-β-cyclodextrin (HP-β-CyD) covered through the adsorption and weak interaction.[27] In this study, we evaluated the ability of C_{60}/HP-β-CyD naonparticles to generate ROS after photoirradiation by using spectroscopic methods including the electron spin resonance (ESR) spin-trapping method. In addition, we examined the photoinduced cytotoxicity of C_{60}/HP-β-CyD nanoparticles using Hela cells, and these photodynamic activities were compared with those of C_{60}/polyvinylpyrrolidone (PVP) particles.

MATERIALS AND METHODS

Materials

C_{60} (nanom purple SUH) was obtained from Frontier Carbon Company (Tokyo, Japan). HP-β-CyD (degree of substitution of 2-hydroxypropyl group is 5.6) was donated by Nihon Shokuhin Kako Company. Ltd. (Tokyo, Japan). PVP (K-30) was obtained from BASF Japan Ltd. (Tokyo, Japan). 5,5-Dimethy-1-pyrroline *N*-oxide (DMPO) was purchased from Labotec Company Ltd. (Tokyo, Japan). 4-Hydroxy-2,2,6,6,-tetramethylpiperidine (TEMP-OH) was purchased from Wako Pure Chemical Industries Ltd. (Tokyo, Japan). Superoxide dismutase (SOD) was purchased from Sigma-Aldrich Company. (Tokyo, Japan). Eagle's minimum essential medium (MEM), Dulbecco's modified Eagle's medium, and penicillin–streptomycin were purchased from GIBCO Invitrogen (Tokyo, Japan). Fetal calf serum was obtained from Nichirei (Tokyo, Japan). All other materials and solvents were of analytical reagent grade, and Milli-Q water was used throughout the study.

Preparation of Hydrophilic C_{60} Nanoparticles and C_{60}/PVP Dispersion

C_{60} (15 mg) was ground with HP-β-CyD (60 mg) in a mole ratio 1:2 (guest:host) using an automatic magnetic agitating mortar (MNV-01, AS ONE Company, Tokyo, Japan) for 3 h at 4°C under reduced pressure. The pulverized C_{60}/HP-β-CyD powder was dispersed in water by ultrasonication for 5 min. C_{60}/PVP particles were prepared in the same manner. The resulting dispersions of C_{60}/HP-β-CyD and C_{60}/PVP were syringe-filtered through a filter of 0.2 or 0.45 μm pore size, respectively. C_{60} was also dispersed in water and various solvents by ultrasonicating intact C_{60} for 1 h, then syringe-filtered through a filter of 0.8 μm pore size. Particle sizes of the C_{60} colloidal solutions were determined by a dynamic light scattering machine (DLS-8000HL, Otsuka Electronics Company Ltd., Tokyo, Japan) equipped with He–Ne laser (10 mW) operating at 632.8 nm. DLS measurements were performed at a scattering angle of 90°. The autocorrelation function was analyzed by the cumulant method to obtain the average particle diameter. Concentrations of C_{60} in the colloidal solutions were determined by the method reported previously.[27]

$O_2^{\cdot-}$ Generation Ability of C_{60}/HP-β-CyD Nanoparticles

$O_2^{\cdot-}$ generation from C_{60}/HP-β-CyD nanoparticles by visible light irradiation was measured by the cytochrome c method, that is, aqueous cytochrome c solution (80 μM) was mixed with C_{60} colloidal solution (C_{60} = 80 μM). The resulting solutions were exposed to visible light supplied from a fluorescence lamp (3500 lux, 400–700 nm, 2 cm from the bottom). An increase in the absorbance at 548 nm of the reduced cytochrome c was measured with a spectrophotometer (U-2800A, Hitachi, Tokyo, Japan).