Note

Photocytotoxicity of Water-soluble Fullere

Kazuhiro IRE, Yoshimasa NAKAMURA, Hajime OHIGASHI, Hidetoshi TOKUYAMA,* Shigeru YAMAGO,** and Eiichi NAKAMURA***

Department of Food Science and Technology, Faculty of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606, Japan
*Faculty of Pharmaceutical Sciences, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan
**Department of Synthetic Chemistry and Biological Chemistry, Kyoto University, Kyoto 606, Japan
***Department of Chemistry, School of Science, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

Received January 25, 1996

New water-soluble fullerene carboxylic acids (1 and 2) derived from C_{60} and C_{70} fullerenes, respectively, were examined for photocytotoxicity toward Raji cells (B lymphocyte). These compounds did not show any photocytotoxic effect even at 50 μM, while pheophorbide a showed significant photocytotoxicity at 0.5 μM. Therefore, fullerene derivatives derived from C_{60} and C_{70} would not be practical agents for photodynamic therapy.

Key words: fullerene, pheophorbide a; photocytotoxicity; photodynamic therapy; porphyrin

Photodynamic therapy (PDT) is one of the recent methods for cancer treatment. PDT is based on the photodynamic action of some porphyrin derivatives and their apparently selective retention in tumor tissues. When the porphyrin-containing tumor is irradiated with an appropriate wavelength laser, singlet oxygen is produced, causing tumor destruction. Since current PDT using a hematoporphyrin derivative (HPD) has several limitations, a number of dyes such as phthalocyanine, chlorin e_{6}, bacteriochlorin, and pyropheophorbide have been reported as potential sensitizers in the past several years. However, there are few reports on non-porphyrin types of sensitizers for PDT. Recent intensive studies on water-soluble fullerene derivatives have indicated that fullerenes could serve as useful photosensitive biochemical probes. Since fullerenes are known to have a significant photodynamic action, we investigated the photocytotoxicity of new water-soluble fullerene carboxylic acids 1 and 2 to examine whether fullerenes could be new lead agents for PDT.

Compounds 1 and 2 were derived from C_{60} and C_{70} fullerenes, respectively, by the method reported previously. Although 1 is a single compound, 2 consists of three regio-isomers in the ratio of 7:2:1; only the major isomer is shown in Fig. 1. Both these compounds were soluble in dimethyl sulfoxide (DMSO) up to 2.5 mM. Figure 2 shows the photocytotoxic effect of 1 on Raji cells (B lymphocyte) along with that of pheophorbide a as a positive control. The cell viability (A and B) and viable cell density (C and D) were plotted, respectively, against the drug concentration. Compound 1 was not photocytotoxic even at 50 μM, while pheophorbide a showed significant photocytotoxicity at 0.5 μM. Compound 1 showed potent growth inhibition in darkness at 50 μM as shown in Fig. 2C, suggesting that a significant cellular uptake of 1 occurred by incubating at 50 μM. A high retention of 1 in several tissues in vivo and the high hydrophobicity (log P = 4.5) were also demonstrated because of its high hydrophobicity (log P = 4.5). Quite similar results were obtained in the case of 2 (data not shown); no significant photocytotoxicity was observed at 50 μM.

Recent studies on the mechanism for PDT have revealed that the triplet lifetime of a photosensitizer determines the photocytotoxicity in cells where the viscosity is much lower than that in the fluid solution. On the basis of our experiments, photosensitizers with a longer triplet lifetime than 1 ms have strong photocytotoxic potential, while those with a triplet lifetime shorter than 100 μs had little photodynamic activity. The triplet lifetimes of C_{60}, C_{70}, and pheophorbide a have been reported to be 40 μs, 130 μs, and 1.5 ms, respectively, agreeing well with our hypothesis.

Nakamura et al. have previously reported that 1 exhibited distinct cytotoxicity under light when it existed both in the cell and the medium. In the present experiment, however, we observed almost exclusively in the cells, and not in the medium, because the cells were washed after the treatment with 1. Since the viscosity in the cells was much higher than that of the medium, 1, whose triplet lifetime was very short, did not show any cytotoxic effect in this experiment. When conducting PDT, a patient is injected intravenously with a photosensitizer, and the drug-containing tumor is irradiated with a laser 24–72 h after the injection. Since this interval is indispensable to attain a clear contrast in the drug concentrations between normal and tumor tissues, fullerene derivatives with a triplet lifetime shorter than 100 μs, as are 1 and 2, would not be practical agents for PDT. However, fullerenes might be useful as drug carriers since the cellular uptake of these fullerenes is very high.

Experimental

Synthesis of 2. A solution of the C_{19}-alcohol (a 7:2:1 mixture of three isomers; 20 mg) was refluxed with 1. NII-Electronic Library Service
Fig. 2. Photocytotoxicity of Raji Cells by I (A and C) and by Pheophorbide α (B and D): ○, with Irradiation; ●, without Irradiation.

Cell viability (% to control) is plotted against the concentration of the drug in A and B. Viable cell density (% to control) is plotted against the concentration of the drug in C and D. Each point represents the average from at least duplicate determinations with less than 10% variation.

in preparation), succinic anhydride (20 mg), and 4-dimethylaminopyridine (24 mg) in CH₂Cl₂ (5 ml) was stirred for 11 h at room temperature. 2 N HCl was added to this brown solution, and the mixture was extracted with CH₂Cl₂. The combined organic extracts were dried over Na₂SO₄ and evaporated. Purification by preparative HPLC in a Buckyprep column (Nacalai Tesque, Japan), using CH₂Cl₂ as the mobile phase, afforded C₁₀₋₁₆-carboxylic acid 2 in 26% yield as a 7:2:1 mixture of three isomers (8.9 mg). IR νmax (CHCl₃) cm⁻¹: 3370, 1733, 1372, 1259, 1100, 1020, 533, 517. NMR δ(CDCl₃, 400 MHz): 0.98 (0.3H, s), 1.01 (0.6H, s), 1.02 (0.6H, s), 1.07 (2H, s), 1.08 (2H, s), 1.26 (3H, s), 2.69 (1H, s), 2.73 (2H, s), 2.85 (2H, s), 3.03 (7H, s), 3.07 (2H, s), 3.25 (0.7H, m), 3.26 (0.7H, s), 3.30 (0.7H, s), 3.37 (1H, m), 3.42 (0.7H, m), 3.71 (2H, s), 3.95 (0.2H, s), 4.00 (0.2H, s), 4.03 (1H, s), 4.07 (1H, s). FAB-MS (matrix, 3-nitro-benzyalcohol) m/z: 1113 (M⁺).

Synthesis of I. The synthesis of I was carried out from the corresponding C₁₀₋₁₆-alcohol in a manner similar to that described for the synthesis of 2. IR νmax (CHCl₃) cm⁻¹: 3045, 2840, 2830, 1735, 1600, 1180, 1160, 1000, 670. NMR δ(CDCl₃, 400 MHz): 1.06 (6H, s), 2.72 (4H, m), 3.62 (2H, d, J = 7.3 Hz), 4.04 (2H, s), 4.14 (2H, s), 4.42 (1H, t, J = 7.3 Hz). FAB-MS (matrix, 3-nitro-benzylalcohol) m/z: 978 (M⁺).

Photocytotoxicity test. Ten milliliters of Raji cells (5 × 10⁶ cells/ml) maintained in RPMI 1640 were dispensed into 15-ml centrifugation tubes. These were centrifuged for 5 min at 1500 rpm with a Hitachi SCT4BE centrifuge. The cells were washed with 5 ml of phosphate-buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na₂HPO₄, and 1.47 mM KH₂PO₄), centrifuged, and the supernatant was removed. Ten milliliters of serum-free RPMI 1640 were then added to the cells. Fifty microliters of a drug in a DMSO solution were added to the solution, which was then vortexed and incubated at 37°C for 30 min in darkness. After washing with 5 ml of PBS and subsequent centrifugation, 10 ml of RPMI 1640 containing fetal bovine serum was added to the cells. The solution was vortexed, and a 2-ml portion was transferred to each 3.5-cm diameter Petri dishes. Two of four dishes were irradiated for 5 min (10 mW/cm²) with light supplied from a PICL-SX (NIPPON P.I. Co., Ltd.) cold spot fitted with a halogen lamp (150 W) and two glass-fiber light guides. After incubating for 48 h in darkness, the cells were treated with Trypan Blue (0.2% in PBS containing 0.2% NaCl), and the viable cells were counted. Control cells were treated in the same way as above without irradiation. The irradiation itself did not cause any hyperthermic effect.

Acknowledgments. This work was supported in part by Grants-in-Aid for Scientific Research for the Encouragement of Young Scientists (No. 07760118) from the Ministry of Education, Science, and Culture of Japan (for K.I.), from Kurata Foundation (for S.Y.), and from Sumitomo Foundation (for E.N.). The authors thank Hoechst Japan for the generous supply of C₇₀.

References
Photocytotoxicity of Water-soluble Fullerene Derivatives