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Non-toxic phototriggered gene transfection by PAMAM-porphyrin conjugates

Ming-Jium Shieh [a,b], Cheng-Liang Peng [a], Pei-Jen Lou [c], Chieh-Hua Chiu [a], Tsiao-Yu Tsai [d], Chia-Yen Hsu [d], Chen-Yu Yeh [d], Ping-Shan Lai [d,*]

[a] Institute of Biomedical Engineering, College of Medicine and College of Engineering, National Taiwan University, No. 1, Section 1, Jen-Ai Road, Taipei 100, Taiwan
[b] Department of Oncology, National Taiwan University Hospital and College of Medicine, No. 7, Chung-Shan South Road, Taipei 100, Taiwan
[c] Department of Otolaryngology, National Taiwan University Hospital and College of Medicine, No. 7, Chung-Shan South Road, Taipei 100, Taiwan
[d] Department of Chemistry, National Chung Hsing University, No. 250, Kuo-Kuang Road, Taichung 402, Taiwan

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ABSTRACT

Development of controllable and non-toxic gene transfection systems is a core issue in gene therapy. Photochemical internalization, an innovative strategy in cytosolic release, provides us with an opportunity to develop a light-inducible gene delivery system. In this study, a novel photochemical internalization (PCI)-mediated gene delivery system was synthesized by surface modification of polyamidoamine (PAMAM) dendrimers via 5,10,15-tri(4-acetamidophenyl)-20-mono(4-carboxyl-phenyl)porphyrin (TAMCPP) conjugated to the generation 4 PAMAM dendrimer (G4). This water-soluble PAMAM-TAMCPP conjugate was characterized for cell viability, phototoxicity, DNA complexation, and *in vitro* transfection activity. The results show that TAMCPP conjugation did not increase the cytotoxicity of the PAMAM dendrimer below 20 μM, but significantly induced cell death after suitable irradiation. Under almost non-toxic G4-TAMCPP-mediated PCI treatment, the expression of green fluorescent protein determined by flow cytometry could be markedly enhanced in HeLa cells. Therefore, the G4-TAMCPP conjugate had an inducible and effective gene transfection activity, and showed considerable potential as a bimodal biomaterial for PCI-mediated gene therapy.

1. Introduction

Gene therapy is a promising strategy to deliver desired gene into target cells for the treatment of genetic deficiencies. Viral vectors have been applied in gene delivery because of their high transfection efficacy. However, their safety concerns, including mutagenesis and immunogenicity, affect their broad application in the clinic [1,2]. Non-viral carriers, such as cationic polymer, have been developed as an alternative delivery strategy with less immunogenicity and lower cost [3,4]. Among these non-viral vectors, polyamidoamine (PAMAM) dendrimer, a novel and unique synthetic macromolecule with a 3-dimensional highly branched structure, is widely used in gene delivery [5,6]. The capability of PAMAM dendrimers to transfect cells appears to depend on the generation and number of primary amino groups on the surface of the polymer. However, generation-dependent cytotoxicity of PAMAM dendrimers has been shown in previous studies [7,8]. Thus, the development of PAMAM dendrimers with low generation, low toxicity, and high transfection efficiency is an important area of research in dendrimer-based gene delivery.

Many studies have demonstrated that surface modification by hydrophobic moieties, such as fluorescent dyes and amino acids, affect the oligonucleotide or gene transfection activity of PAMAM dendrimers [9,10]. Although an enhanced activity can be observed with these systems, the transfection process cannot be controlled by a designed switch. Conjugating an appropriate amount of functionalized hydrophobic molecules to the PAMAM dendrimer to trigger the transfection process and elevate its transfection activity may provide a powerful tool for gene therapy.

Photodynamic therapy (PDT) is a photochemical process for producing localized tissue necrosis, which involves the activation of a photosensitizing drug in the target tissue with light of a specific wavelength matched to an absorption peak of the photosensitiser in the presence of molecular oxygen [11]. It is a recognized therapeutic modality, which has regulatory approval for the treatment of a variety of human pre-malignant and malignant diseases. Photochemical internalization (PCI), a specific branch of PDT, is a novel technology utilized for the site-specific release of macromolecules within cells. The mechanism of PCI is based on the breakdown of the endosomal/lysosomal membranes by photoactivation of photosensitizers that localize on the membranes of these organelles [12]. The PCI strategy has been utilized to release macromolecules such as toxins, DNA delivered as a complex with cationic polymers or incorporated in adenovirus or adeno-associated virus, dendrimer-doxorubicin conjugates, peptide nucleic acids, and bleomycin, from endocytic vesicles to the cytosol [13–19]. Furthermore, PCI can reverse the adriamycin-resistance of breast cancer cells [20].

* Corresponding author. Tel.: +886 4 22840411x409; fax: +886 4 22367269.
E-mail address: f89548003@gmail.com (P.-S. Lai).

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To develop an inducible and low toxic vector, we used a generation 4 PAMAM dendrimer as a gene carrier, and implemented the PCI strategy by conjugating a hydrophobic porphyrin to facilitate its cytosolic release and improve the transfection activity. The cytotoxicity, phototoxicity, and efficacy of the resulting complex were evaluated using HeLa cells as a model, and using the MTT assay and transfection experiments. Our results show that the gene transfection activity of the PAMAM-porphyrin conjugate can be controlled by irradiation under non-toxic conditions, and can be considered as a novel PCI-mediated gene delivery system.

2. Materials and methods

2.1. Materials

Generation 4 polyamidoamine (PAMAM) dendrimer was purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI) and all other chemicals were from Sigma Chemical Co. (St. Louis, MO).

2.2. Synthesis of meso-substituted porphyrins and PAMAM-porphyrin conjugate

5,10,15-tri(4-acetamidophenyl)-20-mono(4-carboxyl-phenyl)porphyrin (TAMCPP) was synthesized from a binary mixture of aldehydes and pyrrole using a modification of the Alder-Longo method as showed in Scheme 1 [21]. ^{1}H NMR (400 MHz, D6-DMSO): δ (ppm) 8.86 (pyrrole, *s*, 6H); 8.81 (pyrrole, *s*, 2H); 8.38 (benzoic acid, *d*, *J*=6.8 Hz, 2H); 8.23 (benzoic acid, *d*, *J*=8.4 Hz, 2H); 8.12 (aniline, *d*, *J*=7.6 Hz, 6H); 8.04 (aniline, *d*, *J*=7.6 Hz, 6H); 2.21 (NH(C=O)CH$_3$, *s*, 9H).

In order to conjugate TAMCPP to the PAMAM dendrimer, TAMCPP was first activated by dicyclohexylcarbodiimide (DCC) and *N*-hydroxysuccinimide (NHS) and yielded the desired TAMCPP-NHS. PAMAM dendrimer-TAMCPP conjugates (G4-TAMCPP) were prepared by reacting G4 PAMAM dendrimers with TAMCPP-NHS. After 48 h at room temperature, the reaction mixtures were dialyzed for another 48 h using a molecular weight cut-off (MWCO) of 10,000 membranes successively against hydrochloric acid/aqueous/methanol solution (0.5/49.5/50). The red conjugate solutions were further concentrated under reduced pressure at 35 °C and the resulting solutions were frozen in liquid nitrogen and lyophilized to obtain the conjugate.

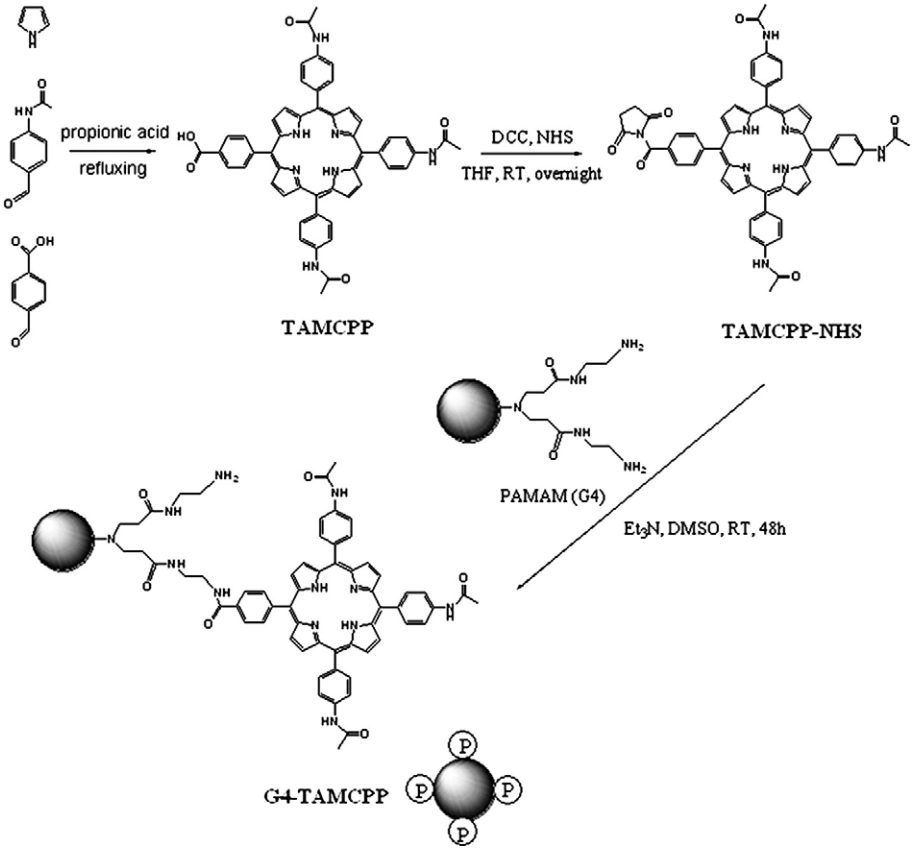
The conjugate was characterized using thin layer chromatography (TLC) and the degree of porphyrin conjugated to PAMAM dendrimer was estimated as 12% (w/w) by a Cary 50 spectrophotometer (Varian Inc., Palo Alto, CA, USA) using the soret molar extinction coefficients of TAMCPP (logε ~5.62 at 420 nm). The lipophilic and hydrophilic properties of G4-TAMCPP were characterized by the partition coefficient $P_{o/w}=C_o/C_w$ of the compound between the two immiscible solvents *n*-octanol (o) and water (w) [22]. The morphology of G4-TAMCPP was observed by transmission electron microscopy (TEM) (Hitachi H-7500) operating at 120 kV.

2.3. Cell lines and culture conditions

Human cervical epithelioid carcinoma (HeLa) cells were maintained in a humidified 5% CO_2 incubator at 37 °C in DMEM (Gibco BRL, Gaithersburg, MD) supplemented with 10% heat-activated fetal bovine serum (FBS) (Gibco BRL, Gaithersburg, MD) and 1% antibiotics (Antibiotic-Antimycotic, Gibco BRL, Gaithersburg, MD, USA).

2.4. Cellular uptake of photosensitizing agents

Cellular uptake of TAMCPP or G4-TAMCPP was measured by fluorometry. Cells (1.5×10^5) were incubated with one of the



Scheme 1. Schematic representation of TAMCPP and G4-TAMCPP conjugate synthesis.