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Dexamethasone conjugated polyallylamine: Synthesis, characterization, and in vitro transfection and cytotoxicity

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ABSTRACT

Nuclear transportation of genetic materials is a critical step in gene therapy. Human gene therapy is not routine enough in clinic because an effective and safe gene carrier does not exist yet. It is reported that glucocorticoids such as dexamethasone (Dexa) facilitate nuclear transportation of plasmid DNA or other polynucleotide molecules to the nucleus. This study is aimed to investigate the influences of Dexa on physicochemical properties, cell transfection and toxicity of polyallylamine (PAA). After activation, Dexa mesylate was conjugated to both unmodified PAA and hexyl acrylate-conjugated PAAs (PAA-6c Acr) through a one-step reaction. PAA-Dexa was purified with dialysis against distilled water and then freeze-dried. The physicochemical properties of the synthesized vectors such as buffering capacity, DNA condensing ability, size, and zeta potential were determined. The PAA 15 kDa-based polyplexes showed better cell transfection than those based on PAA65 kDa in Neuro2A cell line. To sum up, transfection activity was improved in two types of the modified vectors including PAA15-6c Acr30%-Dexa5% at lower carrier to pDNA (C/P) ratios and PAA15-6c Acr50%-Dexa10% at higher C/P ratios. Altogether, the modification of PAA and PAA-6c Acr with Dexa significantly reduced their cytotoxicity, but had not guided their cell transfection to a desired point.

1. Introduction

With promising results, gene therapy showed high potential in treatment of various diseases. However, the deficiency of efficient and safe gene carriers has hindered the routine clinical implementation of human gene therapy [1]. Naked plasmid DNA (pDNA) is rapidly degraded by serum nucleases. Therefore, the need for gene carriers, protecting polynucleotide molecules from degradation, is quite obvious. Nucleic acid (NA) carriers facilitate cellular uptake and transfer of NAs into cytoplasm and nucleus [2].

Gene carriers are primarily categorized into viral and non-viral, both of which have some advantages and limitations. Viral vectors are genetically modified owing to eliminate their pathogenicity as well as retaining their gene transfer potency. They are immunogenic, difficult to manufacture and scale up, as well as size limitation for the inserted NAs [3]. These drawbacks are less encountered in the non-viral gene carriers including cationic lipids (liposomes),

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cationic polymers, and cationic peptides, while their transfection potency is less than that of the viral vectors. Moreover, the physical characteristics such as size, shape, and surface charge will directly influence on their transfection potency. In either gene delivery systems, the chemical modifications, producing safe, efficient, and targeted gene carriers, are more desirable [4–6].

Gene carriers are faced to three major obstacles including cell membrane, endosomal membrane, and nuclear membrane which should be circumvented [7]. Nuclear transportation of pDNA, one of the main hindrances of transfection process, is a critical step in transgene expression. Many strategies have been developed to transfer pDNA to nucleus, one of the most successful of which is incorporation of nuclear localization signals (NLSs) like glucocorticoids (GCs) into carriers [8,9].

Using glucocorticoids as NLS could enhance transgene expression. Glucocorticoid receptor (GR) is an intracellular receptor, associating with heat shock proteins (HSPs) in its inactive form. In the presence of GC, GR detaches from HSP and binds to GC resulting in translocation to nucleus [10]. Furthermore, the nuclear pore complex (NPC) dilated up to 60 nm through this process to expedite transfer of pDNA to nucleus [11]. The potential of GCs to act as NLS had been investigated in many studies [12,13]. It was reported that the improving capacity of the GC-related vectors in transgene expression is linearly correlated to the potency of GC to interact with GR. The higher the potency of GC incorporated into polymer, the more cell transfection will be observed [14].

As a cationic polymer with high amount of primary amine groups on its surface, polyallylamine (PAA) was frequently utilized as a nonviral gene carrier. Despite the capability NA compaction to nano size particles, PAA has some drawbacks as gene carrier including its cytotoxicity and low buffering capacity [15,16]. It was reported that parallel use of lysosome lysing agents like chloroquine increased transfection activity of cationic polymers, but *in vivo* use of such toxic agents is not possible [17]. Thus, it seems reasonable to modify PAA to enhance its buffering capacity and endosomal escape capability as well as ameliorating its toxicity and transfection.

In this study, new derivatives of PAA were developed by conjugation of dexamethasone (Dexa) to PAA to study the influence of this modification on physicochemical characteristics of PAA as well as improve cytotoxicity and transfection properties of it. To this end, the amine groups of PAA 15 and 65 kDa were substituted with 5 or 10% Dexa. The previous study showed that the modified vector composed of PAA 15 kDa, modified with hexyl acrylate (PAA15-6c Acr) in 50% grafting, is a suitable vector for gene delivery [18]. Therefore, besides the unmodified PAAs, PAA15-6c Acr30% and PAA15-6c Acr50% were utilized to be conjugated with Dexa.

2. Materials and methods

2.1. Materials

PAA with different molecular weights (15 and 65 kDa), [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] (MTT), and Dulbecco's Modified Eagle Medium (DMEM) were supplied by Polyscience Inc. (USA). Dexa base was supplied by aburaihan pharmaceutical Co. (Iran). Hydrochloric acid, Sodium hydroxide, Ethyl acetate, Dimethyl Sulfoxide (DMSO), and anhydrous Pyridine were purchased from Merck (Germany). 2,4,6-trinitrobenzene sulfonic acid (TNBS), Sodium tetraborate ($Na_2B_4O_7.10H_2O$) (Borax), Methanesulfonyl chloride, and ethidium bromide (EtBr) were purchased from Sigma-Aldrich (Germany). 2-iminothiolane (Traut's reagent) was supplied by ThermoFisher Scientific (Pierce, USA). PAA15-6c Acr30% and PAA15-6c Acr50% were previously synthesized [18]. Plasmid DNA, containing *EGFP* gene regulated with the cytomegalovirus enhancer/promoter was purchased from Promega (Madison, WI, USA). All of the solvents and buffers (with the highest purity) were provided by Sigma-Aldrich (Germany).

2.1.1. Preparation of plasmid DNA

Plasmid DNA (encoding EGFP) was cloned in *Escherichia coli*-DH5α and extracted by QIAGEN Mega Plasmid kit according to the manufacturer's protocol.

2.2. Cell culture

Neuro2A cells (neuroblastoma cells, ATCC CCL-131) were cultured in DMEM, supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin, and 100 μg/ml streptomycin. All cells were incubated at 37 °C in a humidified atmosphere containing 5% CO_2.

2.3. Synthesis of Dexamethasone–PAA (Dexa–PAA)

2.3.1. Activation of Dexa

In order to activate Dexa, the 21-hydroxyl group was replaced with mesylate group. Methanesulfonyl chloride (50.17 μl, 0.644 mmol) was added dropwise to the solution of Dexa (210.36 mg, 0.536 mmol) in 4 ml of anhydrous pyridine at 0 °C with stirring under continuous N_2 gas stream. The reaction was done for 5 h at 0 °C, and then ice water (40 ml) was added to halt the reaction. The white precipitate was filtered and washed with excess amount of ice water. Thereafter, the crude dexamethasone mesylate (Dexa-mesylate) was yielded and dried. To confirm the synthesis of Dexa-mesylate, TLC (ethyl acetate/hexane 1/1, v/v) was performed.

2.3.2. Conjugation of PAA and Dexa-mesylate

Traut's reagent and Dexa-mesylate (both 4 equiv.), dissolved in 2.0 ml of anhydrous dimethyl sulfoxide (DMSO), were added slowly to 1 equiv. of PAA in 1 ml anhydrous DMSO under N_2 gas stream at room temperature with continuous stirring for 4 h. To halt the reaction, cold ethyl acetate was added. The harvested product was dissolved in water, and then dialyzed against pure water with dialysis membrane (MWCO 1000) for 48 h, the dialysis medium was refreshed every 12 h. The final product was lyophilized to achieve Dexa-modified PAA (PAA-Dexa) powder.

2.4. Determining the Dexa grafting percentage

The amount of primary amine groups of the modified PAAs was specified by quantification of free primary amines through coupling with 2,4,6-trinitrobenzene sulfonic acid (TNBS) [19]. Briefly, 20 μl of fresh TNBS solution (15 mg/ml) was added to 96-well microplate containing various amounts of PAA (dissolved in 600 μl of double distilled water (ddH_2O)). The mixtures were diluted by 200 μl of sodium bicarbonate buffer solution (0.8 M and pH 9.2) and the UV absorbance was measured at 410 nm.

2.5. Measuring the buffering capacity of modified PAAs

PAA derivatives (0.4 mg/ml) were dissolved in ddH_2O and pH was adjusted to 12. Then 5 μl of HCl (1 N) was added stepwise and pH was measured. The addition of HCl was continued until pH was reached to 2.5 and pH of each step was measured. The resultant diagrams, plotting the amount of the added HCl versus pH, were utilized to find out any change in the buffering capacity of the modified PAAs.