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ORIGINAL ARTICLE

DISTRIBUTION OF POLYETHYLENIMINE IN ZEBRAFISH EMBRYOS

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ABSTRACT

Background. Polyethylenimine (PEI) plays important roles in the pharmaceutical design of non-viral gene delivery systems. Due to a set of unique physicochemical properties this cationic polymer has a great potential in modern gene therapies.

Objective. The aim of the present study was to determine the distribution of branched PEI (0.8 kDa) in zebrafish embryos *(Danio rerio)*.

Material and methods. Zebrafish embryos at 3 hours post-fertilization (hpf) were incubated with PEI (10 μg/ml) for 24 and 48 hours and studied using the confocal laser microscopy.

Results. The obtained results show that PEI effectively distributed into the layers of the chorion and yolk sac in developing embryos due to first 24 hours of exposure. In contrast, PEI was found in the yolk, head, trunk and tail of the embryos due to prolonged treatments (48 hours).

Conclusion. The study evidences a high distribution of the branched PEI (0.8 kDa) polymer in the zebrafish embryo tissues.

Key words: *polyethyleneimine (PEI), distribution, zebrafish embryos.*

STRESZCZENIE

Wprowadzenie. Polietylenoimina (PEI) odgrywa ważną rolę w projektowaniu niewirusowych transporterów genów. Ze względu na unikalne właściwości fizykochemiczne, ten kationowy polimer posiada duży potencjał aplikacyjny w nowoczesnych terapiach genowych.

Cel badań. Celem niniejszego badania było określenie dystrybucji rozgałęzionego PEI (0,8 kDa) w zarodkach Danio pręgowanego *(Danio rerio)*.

Materiały i metodyka. Trzy godziny po zapłodnieniu zarodki Danio pręgowanego inkubowano z PEI (10 μg/ml) przez 24 godziny i 48 godzin po czym badano przy użyciu konfokalnej mikroskopii laserowej.

Wyniki. Wykazano, że PEI ulega dystrybucji w warstwach chorionu oraz żółtku rozwijającego się zarodka w pierwszych 24 godzinach ekspozycji. W wyniku przedłużonej ekspozycji (48 godzin), wykazano obecność PEI w żółtku, głowie, tułowiu i ogonie zarodków.

Wnioski. Badania wskazują na znaczącą dystrybucję rozgałęzionego polimeru PEI (0,8 kDa) w tkankach zarodka Danio pręgowanego.

Słowa kluczowe: *polietylenoimina , PEI, dystrybucja, zarodki Danio pręgowanego*

INTRODUCTION

Polyethyleneimine (PEI) is a synthetic polymer, which has been recently used in novel non-biological gene delivery systems [16]. Studies evidence that PEI forms stable complexes with DNA, and the binding is achieved mainly due to direct interaction between the protonated imino groups on PEI and the electronegative oxygens on the DNA backbone [4]. The high density of secondary and tertiary amino groups in PEIs confers significant buffering capacity to the polymers over a wide pH range [15]. This property known as

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"proton sponge effect" is mainly recognized as one of the crucial factors for the high transfection efficiency due to PEI associated cargos [2].

Recent studies elucidate that PEI mediated toxicity depends on its molecular weight and type of the PEI structure [9]. The low molecular weight linear or branched PEI has low cytotoxicity compared to its high molecular weight counterparts [6]. Moreover, the linear PEIs are more tolerable than the branched ones [5]. Generally, it is assumed that the backbone linkages (carbon-carbon or carbon-amine bonds) in branched PEIs are non-degradable at physiological pH and are resistant to systemic clearance and accumulate in cells leading to further toxicities [12]. Because using branched PEIs as non-viral vectors for different nucleic acid cargos is very challenging, a more thorough understanding of the whole-body fate of these cationic polymers is really needed.

In the present study, we evaluated the distribution of branched polyethylenimine in zebrafish embryos *(Danio rerio)*. Both early and late stages of embryonal developments were studied due to PEI exposures.

MATERIAL AND METHODS

Reagents

Branched polyethylenimine (PEI; Mw (by LS): 0.8 kDa), fluorescein isothiocyanate (FITC), dimethyl sulfoxide (DMSO) and tricaine were purchased from Sigma-Aldrich (Germany). All the reagents were used as received without purification. Dialysis against water was carried out using 0.5 k MCWO Snake-Skins Dialysis Tubes (Thermo-Fisher Scientific, MA USA). Lyophilization was performed using a FreeZone 1 liter Laboratory Lyophilizer (Labconco, MO USA). All manipulations were made in the dark with a red bulb.

Synthesis of PEI-FITC labels

Branched PEI-FITC derivatives were synthesized using the methods described elsewhere with slight modifications [10, 13]. To a stirred solution of PEI (0.8 kDa; 140 mg) in distilled water (10 ml) a solution of FITC (11.2 mg; 28.7 μmol) in DMSO (5 mL) was added. The reaction mixture was stirred at room temperature for 24 hours and then dialyzed (0.5k MWCO membrane for PEI 0.8 kDa) against distilled water for 48 h. Finally, after lyophilization for 24 h *ca*. 70 mg of the final product (PEI-FITC) was obtained respectively.

Animals

Zebrafish embryos *(Danio rerio)* of the AB strain were obtained from the International Institute of Molecular and Cellular Biology (Zebrafish Core Facility) in Warsaw (Poland). In brief, adults zebrafish (male and female) were kept at the standard laboratory condition of 28.5°C on a 14 hours light/10 hours dark photoperiod in the so-called zebrafish water. After photo-inducted spawning of ten pairs (male and female; ratio 1:1), the embryos were collected and staged as described by Kimmel et al. [11]. Well-developed zebrafish embryos at the 8-32 cellular stage were selected under a dissecting microscope and used for the distribution studies.

Zebrafish embryo exposures to PEI

The zebrafish embryos at 3 hpf were incubated with a water solution of PEI labeled with FITC at 10 μg/ml for 24 and 48 hours, respectively. The embryos treated with ultrapure water were served as controls. To data, the exposures were performed using 96-well plates with one embryo in each well. The embryos were held continuously under a 14:10 light/dark photoperiod at 28.5 ± 0.5°C.

Confocal microscopy studies

Randomly selected zebrafish embryos from 24 and 48 hours exposures were transferred on a petri dish and rinsed with zebrafish medium [1]. In the case of the embryos at 24 hours exposure, the study was performed with embryos possessing with and without the chorions. The embryos were then transferred in a drop of the zebrafish medium into 96-well plate (one embryo per one well) and anesthetized with a drop of tricaine. Each anesthetized embryo was covered with melting agarose (42°C) and immediately positioned on its side for imaging from the lateral side. The embedded embryos were imaged with an Olympus X1000 confocal microscope. Images were taken with 10X objective and excitation wavelength 488 nm. Fluorescence signal was acquired together with DIC imaging.

RESULTS AND DISCUSSION

The zebrafish species are the most frequently used animal models in modern toxicological studies [3]. It owes its great popularity due to the availability of a large number of individuals at the embryonic development, low cost, transparency, susceptibility to genetic manipulation and usefulness in high-throughput screening research [14]. In the present study, the distribution of non-modified branched polyethylenimine (0.8 kDa) was examined using the embryonic zebrafish model *(in vivo)*. To data, PEI was conjugated with FITC and used in the study in zebrafish embryos. The results evidence the substantial accumulation of the PEI material in the layers of the chorion resulting in the inhibition of the hatching process in the early embryos (Figure 1).

Interestingly, PEI also distributes into the tissues of the developing embryos because the increase of fluorescence intensities was noted in the yolk sac due to dissection of the chorion (Figure 2).

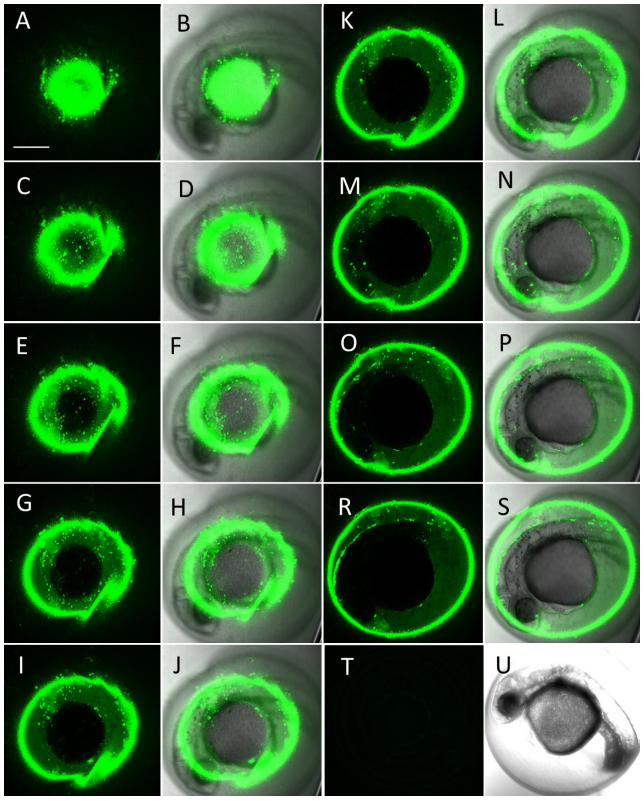

Figure 1. Distribution of PEI 0.8 kDa within the chorion of the *Danio rerio* embryo after 24 hours of exposure (10 µg). Serial optical sections (z-stacks, 24 µm/slice) of the confocal images of exposed (A-S) and non-exposed (T-U) embryos. Florescence measurements (green, A-U) indicate localization of the PEI polymer. Fluorescent signal is also merged with DIC (differential interference contrast) (grey, B,D,F,H,J,L,N,P,S,U). Scale bar 300 µm.

In recent studies, polyethylenimine was shown to have a high affinity for parenchymal cells, especially the lung, liver, spleen, kidney, heart and brain of laboratory mice [8]. After intraventricular injection of the PEI-DNA complex into the cerebrospinal fluid of mice, a good cerebral distribution of this complex was also observed [7]. In other studies, the PEI-DNA complex were mainly deposited in zebrafish embryos due to short-term exposures [17]. In the present study, the distribution of PEI was found within the yolk sac and head of the developing embryo due to first 24 hours of exposures (Figure 2). Interestingly, PEI was mainly determined in the yolk, trunk and head of embryos due to prolonged (48h) exposures (Figure 3).

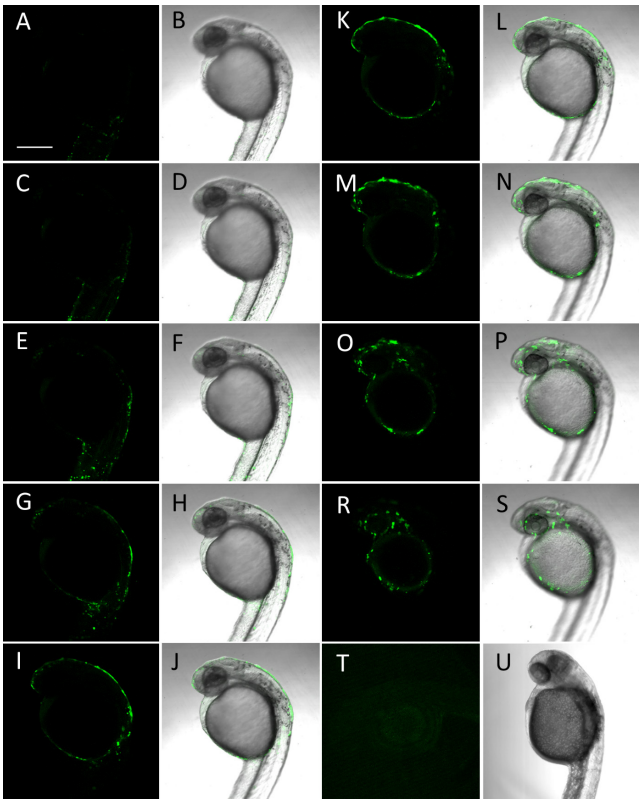

Figure 2. Distribution of PEI 0.8 kDa in the yolk sac and head in the dechorionated zebrafish embryo after 24 hours of exposure (10 µg). Serial optical sections (z-stacks, 33 µm/slice) of confocal images of treated (A-S) and non-exposed (T-U) embryos. Florescence (green, A-U) indicates localization of PEI and fluorescent signal merged with DIC imagining of embryo (grey, B,D,F,H,J, L,N,P,S,U). Scale bar 300 µm.

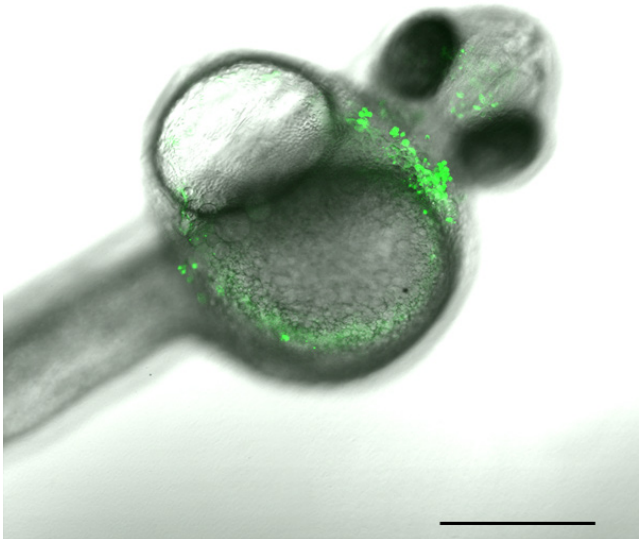

Figure 3. Distribution of PEI 0.8 kDa in the head and yolk sac in the dechorionated zebrafish embryo after 48 hours of exposure (10 µg). The yolk sac edema is found. Scale bar 300 mm.

CONCLUSION

The study evidences that PEI easily distributes into all organs and tissues in zebrafish embryos. A large body of PEI is found at the chorion, yolk sac and head of the growing embryos due to 24- and 48-hours exposures.

Acknowledgments

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Conflict of interest

The authors declare that there are no conflicts of interest.

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