Data Article

Data on synthesis and characterization of chitosan nanoparticles for in vivo delivery of siRNA-Npr3: Targeting NPR-C expression in the heart

Balaji Venkatesan, Anusha Tumala, Vimala Subramanian, Elangovan Vellaichamy*

Department of Biochemistry, University of Madras, Guindy campus, Chennai 600025, India

**Article info**

**Article history:**
Received 21 April 2016
Received in revised form 18 May 2016
Accepted 30 May 2016
Available online 3 June 2016

**Keywords:**
Chitosan nanoparticles
Gene silencing
Biocompatibility
Hemocompatibility

**Abstract**

This data article contains the data related to the research article 'Transient silencing of Npr3 gene expression improved the circulatory levels of atrial natriuretic peptides and attenuated β-adrenoceptor activation-induced cardiac hypertrophic growth in experimental rats' (Venkatesan et al., 2016 [1]). The siRNA-Npr3 loaded chitosan nanoparticles were synthesized using ionotropic gelation method, where the positive charge of the chitosan interacts with the negative charge of STPP and siRNA-Npr3. The physicochemical properties of the synthesized siRNA-Npr3 loaded chitosan nanoparticles were studied by dynamic light scattering, FE-SEM and HR-TEM analysis. In addition, the loading efficiency and stability of the nanoparticles were also studied. Further, the gene silencing efficacy, hemocompatibility and biocompatibility were studied using Wistar rats (in vivo), isolated red blood cells and H9c2 cardiomyoblast cells, respectively.

© 2016 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).
**Specifications Table**

| Subject area | Biology |
|--------------|---------|
| More specific subject area | Nanotechnology, Molecular biology. |
| Type of data | Figure. |
| How data was acquired | FE-SEM, HR-TEM, DLS, Zeta potential, agarose gel electrophoresis, hemocompatibility assay, MTT assay, RT-PCR and Western blotting. |
| Data format | Raw and analyzed. |
| Experimental factors | Synthesis of siRNA-Npr3 loaded chitosan nanoparticles by ionotropic gelation. |
| Experimental features | Hypertrophied H9c2 cells and hypertrophied rats were treated with different concentration of target 1 and 2 siRNA-Npr3 loaded chitosan nanoparticles to validate its gene silencing efficacy. |
| Data source location | NA. |
| Data accessibility | Data are available within this article. |

**Value of the data**

- This data describes the synthesis and characterization of the siRNA-Npr3 loaded chitosan nanoparticles for in vitro and in vivo applications.
- This data validated the biocompatibility, hemocompatibility and gene silencing efficacy of siRNA-Npr3 loaded chitosan nanoparticles in *in vitro* and *in vivo* model system.
- This method of siRNA-Npr3 loaded chitosan nanoparticles can be utilized as a drug delivery vehicle for *in vitro* and *in vivo* applications.

**1. Data**

The data provided here displays the synthesis, characterization, biocompatibility and hemocompatibility of siRNA-Npr3 loaded nanoparticles. Further, the gene silencing efficacy of the synthesized siRNA-Npr3 nanoparticles was demonstrated in the H9c2 cells *in vitro* and in rat hearts *in vivo*.

**2. Experimental design, materials and methods**

**2.1. Materials**

Chitosan (75–85% of deacetylation, low molecular weight), Npr3 specific siRNA – Target 1: 5’-GUUUUCUAAUGGCCUUCUC[dT][dT]-3’ and 5’-UAGAAGGCCAUUACAAAC[dT][dT]-3’ (CAT# SASI_Rn01_00055729), Target 2: 5’-GACUAUGCUUUCUCCAA[dT][dT]-3’ and 5’-UGUUGAGAAAGCAUAGUC[dt] [dT]-3’ (CAT# SASI_Rn02_00260355) and sodium tripolyphosphate were procured from Sigma-Aldrich, USA. Dulbecco’s Modified Eagle’s Medium (DMEM), Trypsin-EDTA, fetal bovine serum (FBS) and antibiotic antymycotic solution were purchased from HiMedia, India. cDNA conversion kit was procured from Thermo scientific, USA. Red dye PCR master mix was purchased from Merck Millipore, German. Gene specific primers were purchased from Eurofins Scientific, Luxembourg. Primary antibody for NPR-C and HRP labeled secondary antibody were purchased from Santa Cruz biotechnology, USA.

**2.2. Synthesis and characterization of siRNA-Npr3 loaded chitosan nanoparticles**

The siRNA-Npr3 loaded chitosan nanoparticles were synthesized by mixing chitosan solution (1 mg/ml chitosan in 0.2 M sodium acetate buffer, pH 4.5) to a mixture containing STPP solution
(2.5 mg/ml) and siRNA-Npr3 at 5:1 weight ratio of chitosan to STPP and 50:1 N:P ratio of chitosan and siRNA-Npr3. The contents were mixed and vortexed for 1 min on a vortex mixer and kept undisturbed for 30 min [2]. The synthesized nanoparticles were purified by centrifuging at 31,000 g for 20 min. Further, the nanoparticles were washed with ultrapure DNase/RNase free water and centrifugation was repeated.

Fig. 1A shows the particle size analysis (DLS – Malvern Nano ZS, UK) of synthesized nanoparticles, which revealed that the hydrodynamic size of the siRNA-Npr3 nanoparticles were in the range of 220 ± 17.5 nm. From the zeta potential analysis, the nanoparticles were found to possess a surface charge of +16.2 ± 1.2 mV. It is evidenced from the FE-SEM – Fig. 1B (Hitachi SU6600, Germany) and HR-TEM – insert in Fig. 1B (FEI TECNAI G2) analysis of the nanoparticles that the synthesized particles were of fairly spherical in shape and evenly distributed.

2.3. Quantification of siRNA-Npr3 loading efficiency

The loading efficiency of the siRNA-Npr3 in the siRNA-Npr3 loaded chitosan nanoparticles were analyzed using UV–Vis spectrophotometer (Shimadzu UV 150-02, Japan) by comparing the A260 of the
supernatant solution obtained after the synthesis of siRNA-Npr3 loaded chitosan nanoparticles and the naked siRNA-Npr3 [3]. The loading efficiency was calculated by using the following formula: Loading efficiency = \( \frac{A_{260\text{ nm}}\text{ of siRNA present in the supernatant}}{A_{260\text{ nm}}\text{ of total amount of siRNA added for nanoparticles preparation}} \times 100 \) and the siRNA-Npr3 loading efficiency were observed to 100% (Fig. 1C).

2.4. Gel retardation assay

The stability and interaction strength between the chitosan and siRNA-Npr3 in the nanoparticles was carried out by gel retardation assay. Briefly, equal concentration of naked siRNA-Npr3, chitosan nanoparticles and siRNA-Npr3 loaded chitosan nanoparticles were loaded on different wells of 4% agarose gel for analyzing the stability of the nanoparticles [3]. The gel retardation assay showed that the nanoparticles loaded with siRNA-Npr3 gets retarded in the well as evidenced by the retarded movement of the nanoparticles, while the naked siRNA-Npr3 freely resolved in the agarose gel (Fig. 1D).

2.5. Biocompatibility of siRNA-Npr3 loaded chitosan nanoparticles

To assess the biocompatibility of the nanoparticles, MTT assay was carried out on H9c2 cell line. Briefly, 5000 cells/well were seeded in 96 well plate and maintained in 10% FBS containing DMEM for 24 h. After 24 h, the cells were treated with either chitosan nanoparticles or siRNA-Npr3 loaded chitosan nanoparticles or chitosan scrambled siRNA nanoparticles in serum free media for 48 h. Later, the media was removed and 10 \( \mu \)l of MTT solution (5 mg/ml) was added to each well and incubated for 4 h at dark. After the reaction, the MTT was removed and 100 \( \mu \)l of DMSO was added to all the wells. The \( A_{570} \) was read by microplate reader [4]. Fig. 2A shows the results of the biocompatibility assay, where none of the nanoparticles (chitosan, siRNA-Npr3 loaded chitosan nanoparticles, and chitosan scrambled nanoparticles) tested exhibits cytotoxicity against H9c2 cells.

2.6. Hemolytic activity

The hemolytic activity of the nanoparticles was tested on isolated rat erythrocytes [5]. Briefly, the whole blood was processed and erythrocytes were collected in a vial. 5% v/v erythrocytes in PBS were distributed to each tube and the volume of the tube was made up to 1 ml with nanoparticles samples (chitosan nanoparticles or siRNA-Npr3 loaded chitosan nanoparticles or chitosan scrambled siRNA nanoparticles) and PBS. Erythrocytes treated with 1% triton X 100 served as the positive control and erythrocytes in PBS served as negative control. The reaction mixture was incubated for 1 h at 37 °C in shaking condition. Then the tubes were centrifuged at 190g and the absorbance of the supernatant was measured at 540 nm. The percentage of hemolysis = \( \frac{\left( A_{540\text{ nm}}\text{ in siRNA-Npr3 loaded chitosan nanoparticles supernatant solution} - A_{540\text{ nm in PBS}} \right)}{\left( A_{540\text{ in 1% Triton X-100} - A_{540\text{ in PBS}}} \right) \times 100} \). Fig. 2B, shows the results of the hemolytic activity assay. The nanoparticles (150 \( \mu \)g/ml) were tested individually on rat erythrocytes and found to be exhibit least toxicity as per ASTM standard which can be considered as compatible [6].

2.7. Validation the gene silencing efficacy of siRNA-Npr3 loaded chitosan nanoparticles

The in vivo gene silencing efficacy and effective dosage fixation of siRNA-Npr3 loaded chitosan nanoparticles were performed by intramyocardial injection of the nanoparticles. Briefly, 2.5 or 5 \( \mu \)g of siRNA-Npr3 containing nanoparticles/kg body weight was administered to the hypertrophied Wistar rats. At the end of the experiment, the heart tissue was harvested and processed as described earlier [1]. Fig. 2C and D shows the Western blotting and densitometric analysis of NPR-C protein expression
on the control and experimental group of rat hearts, where a 4 fold increase in the NPR-C protein expression was observed in the isoproterenol treated rat hearts, and treatment with 2.5 or 5 µg of siRNA-Npr3 decreased the expression of NPR-C by 45% and 70% respectively.

To validate the gene silencing efficacy of the siRNA-Npr3 loaded chitosan nanoparticles, H9c2 cells were seeded on 6 well plates (0.15 x 10^6 cells/well) and treated as follows: 1) control cells-no treatment; 2) ISO treated-ISO (10 µM); 3) ISO + 25 nM siRNA-Npr3 (target 1); 4) ISO + 50 nM siRNA-Npr3 (target 1); 5) ISO + 25 nM scrambled siRNA (target 1); 6) ISO + 50 nM scrambled siRNA (target 1); 7) ISO + 25 nM siRNA-Npr3 (target 2); 8) ISO + 50 nM siRNA-Npr3 (target 2); 9) ISO + 25 nM scrambled siRNA (target 2); 10) ISO + 50 nM scrambled siRNA (target 2). At the end of 48 h of treatment, NPR-C expression was analyzed by RT-PCR and Western blotting analysis. The RT-PCR and Western blotting analysis showed a significantly (3-fold) increased Npr3 gene expression on treatment with ISO. Upon co-treatment with siRNA target 1 (25 nM) and target 2 (25 nM), the level of Npr3 gene expression was found to be decreased by 1.5-fold, respectively (Fig. 3A–H).

**Fig. 2.** Biocompatibility, hemocompatibility and targeting efficacy of siRNA-Npr3 loaded chitosan nanoparticles, in vivo: A) cytotoxicity of chitosan nanoparticles on H9c2 cells by MTT assay. Data are represented as mean ± S.E.M. (n = 3), where, a – untreated vs chitosan nanoparticles (Non-significant), b – untreated vs siRNA-Npr3 nanoparticles (Non-significant), B) Representative image of hemocompatibility of the chitosan nanoparticles on erythrocytes where, (a) treatment with 1% triton X 100 (positive control), (b) treatment with chitosan nanoparticles, (c) treatment with siRNA-Npr3 loaded chitosan nanoparticles and (d) treatment with scrambled siRNA nanoparticles. C) Representative immunoblot analysis of NPR-C protein expression on treatment with different dose of siRNA-Npr3, where lane 1 – Control, 2 – ISO-treated for 7 days, 3 – ISO + siRNA-Npr3 nanoparticles (2.5 µg/kg body weight), 4 – ISO + siRNA-Npr3 nanoparticles (5 µg/kg body weight). D) Densitometric analysis of NPR-C immunoblot. Data are represented as mean ± S. E.M. (n=6 rats). *P < 0.01 – Control vs ISO-treated, **P < 0.05 – ISO-treated vs ISO + siRNA-Npr3 (dose 1), ^P < 0.01 – ISO-treated Vs ISO + siRNA-Npr3 (dose 2).
Fig. 3. Validation of siRNA-Npr3 loaded chitosan nanoparticles on H9c2 cells in vitro: A) and B) Representative RT-PCR and densitometry of Npr3 gene expression, respectively. C) and D) Representative immunoblot and densitometry of NPR-C protein expression, respectively, where L-100 bp DNA ladder, Lane 1 – control, 2 – ISO-treated, 3 – ISO+ siRNA target 1(25 nM), 4 – ISO+ siRNA target 1 (50 nM), 5 – ISO+ scrambled siRNA target 1(25 nM), 6 – ISO+ scrambled siRNA target 1(50 nM). Data are represented as mean ± S.E.M. (n = 3). *-P<0.01 – Control vs ISO-treated, ^-P<0.05 – ISO-treated vs ISO+ siRNA target 1(25 nM). #=Non-significant – ISO-treated vs ISO+ scrambled siRNA target 1(25 nM). ##=Non-significant – ISO-treated vs ISO+ scrambled siRNA target 1 (50 nM). E) and F) Representative RT-PCR and densitometry of Npr3 gene expression, respectively. G) and H) Representative immunoblot and densitometry of NPR-C protein expression, respectively, where L-100 bp DNA ladder, Lane 1 – control, 2 – ISO-treated, 3 – ISO+ siRNA target 2(25 nM), 4 – ISO+ siRNA target 2(50 nM), 5 – ISO+ scrambled siRNA target 2(25 nM), 6 – ISO+ scrambled siRNA target 2(50 nM). Data are represented as mean ± S.E.M. (n = 3). *-P<0.01 – Control vs ISO-treated, ^-P<0.05 – ISO-treated vs ISO+ siRNA target 2(25 nM). ***=P<0.01 – ISO-treated vs ISO+ scrambled siRNA target 2(25 nM). **=Non-significant – ISO-treated vs ISO+ scrambled siRNA target 2 (50 nM).
Acknowledgment

Dr. EV would like to thank Indian Council of Medical Research (ICMR: Grant no. 35/27/2010-BMS) and UGC-SAP DSR II (Grant no. F.5-10/2015/DSR-II (SAP-II)) for the financial support. We also like to acknowledge the electron microscopy facilities provided by the National Center for Nanoscience and Nanotechnology, University of Madras, India.

Transparency document. Supplementary material

Transparency document associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.dib.2016.05.074.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.dib.2016.05.074.

References

[1] B. Venkatesan, A. Tumala, V. Subramanian, E. Vellaichamy, Transient silencing of Npr3 gene expression improved the circulatory levels of atrial natriuretic peptides and attenuated β-adrenoceptor activation-induced cardiac hypertrophic growth in experimental rats, Eur. J. Pharmacol. 782 (2016) 44–58.
[2] K.A. Howard, U.L. Rahbek, X. Liu, C.K. Damgaard, S.Z. Glud, M.O. Andersen, M.B. Hovgaard, A. Schmitz, J.R. Nyengaard, F. Besenbacher, J. Kjems, RNA interference in vitro and in vivo using a chitosan/siRNA nanoparticle system, Mol. Ther. 14 (4) (2006) 476–484.
[3] H. Katas, H.O. Alpar, Development and characterisation of chitosan nanoparticles for siRNA delivery, J. Control. Release 115 (2) (2006) 216–225.
[4] Tim Mosmann, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, J. Immunol. Methods 65 (1) (1983) 55–63.
[5] B. Venkatesan, V. Subramanian, A. Tumala, E. Vellaichamy, Rapid synthesis of biocompatible silver nanoparticles using aqueous extract of Rosa damascena petals and evaluation of their anticancer activity, Asian Pac. J. Trop. Med. 7 (2014) S294–S300.
[6] Document ASTM, Standard practice for assessment of hemolytic properties of materials designation: F 756-00. Annual Book ASTM Standard, 2005, pp. 309–313.