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Promoter- and strain-selective enhancement of gene expression in a mouse skeletal muscle by a polymer excipient Pluronic P85

Zhihui Yang [a], Jian Zhu [a,1], Srikanth Sriadibhatla [a], Catherine Gebhart [a,2], Valery Alakhov [b], Alexander Kabanov [a,*]

[a] *Center for Drug Delivery and Nanomedicine and Department of Pharmaceutical Sciences, College of Pharmacy, University of Nebraska Medical Center, 985830 Nebraska Medical Center, Omaha, NE 68198-5830, United States*
[b] *Supratek Pharma Inc, 15 Boul. Bouchard, Suite 1315, Dorval, Quebec, Canada H9S 1A9*

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Abstract

Amphiphilic triblock copolymers of ethylene oxide and propylene oxide (Pluronic) significantly enhanced expression of plasmid DNA in the skeletal muscle. In the presence of Pluronic P85 (P85) high levels of expression of a reporter gene (luciferase) were sustained for at least 40 days and the area under the gene expression curve increased by at least 10 times compared to the DNA alone. The effect of Pluronic depended on the strain of the mouse and the type of the promoter used. Thus, P85 enhanced luciferase expression by 17 to 19-fold in immunocompetent C57Bl/6 and Balb/c mice, while no enhancement was observed with athymic Balb/c nu/nu mice. Furthermore, P85 activated the expression of luciferase gene driven by CMV promoter, NFκB and p53 response elements. There was much less or no effect on the gene driven by SV40 promoter or AP1 and CRE response elements. Overall, the promoter selectivity suggested that Pluronic induced transcriptional activation of gene expression by activating the p53 and NFκB signaling pathways. In addition Pluronic increased the number of DNA copies and thus affected initial stages of gene transfer in a promoter selective manner.

Keywords: Gene expression; Skeletal muscle; Plasmid DNA; Block copolymer; p53; NFκB; Transcription; Inflammation

1. Introduction

Injection of plasmid DNA into skeletal muscle results in gene transfer into myocytes and gene expression in vivo [1]. Intramuscular (*i.m.*) injections of naked DNA were evaluated for the treatment of muscular dystrophies (MDs), local or systemic secretion of therapeutic proteins, and elicitation of immune

* Corresponding author. Center for Drug Delivery and Nanomedicine, Durham Research Center, Room 1036, 985830 Nebraska Medical Center, Omaha, NE 68198-5830. Tel.: +1 402 559 9915; fax: +1 402 559 9365.
E-mail address: akabanov@unmc.edu (A. Kabanov).
[1] Present address: Department of Pharmacology and Molecular Sciences, The Johns Hopkins School of Medicine, 725 N. Wolfe Street, Baltimore, MD 21205 United States.
[2] Present address: Molecular Diagnostics Laboratory, 985454 Nebraska Medical Center, Omaha, NE 68198-5454 United States.

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responses against antigens of infectious diseases or cancer [2–8]. The use of plasmid DNA can avoid inherent problems of recombinant viral vectors, including immune and toxic reactions as well as potential viral recombination [9]. However, the application of this technique is hindered by poor transduction efficiency and high variability of gene expression [10,11]. Cationic lipids and polycations have been developed to enhance DNA delivery. However, the efficiency of these cationic molecules in the muscle is usually quite low, and in many cases less than that of the naked DNA. Conversely, some non-ionic polymer carriers, such as polyvinyl pyrrolidone (PVP), were shown to improve the gene expression of naked DNA in the muscle [12,13]. Another group of non-ionic polymers, called Pluronic block copolymers has shown substantial promise by increasing the gene expression of the plasmid DNA in skeletal and cardiac muscle as well as in solid tumors [14–18]. Pluronics consist of ethylene oxide (EO) and propylene oxide (PO) chains arranged in a triblock structure: $EO_x-PO_y-EO_x$. The present work uses Pluronic P85 (P85) and a mixed Pluronic formulation SP1017 to characterize the promoter selective effects of Pluronics on expression of plasind DNA delivered *i.m.* The promoter selectivity suggests that Pluronics may activate selected signaling pathways, including NFκB and p53, resulting in activation of the transcription of the genes delivered in the muscle.

2. Materials and methods

2.1. Plasmids and reagents

The gWiz™ plasmids (Gene Therapy Systems, San Diego, CA) encoded the luciferase (gWiz™ Luc) and β-galactosidase (gWiz™ β-gal) each under control of an optimized human cytomegalovirus (CMV) promoter followed by intron A from the CMV immediate-early (IE) gene. The pGL3-promoter vector (Promega, Madison, WI) encoded luciferase under the control of SV40 promoter. PathDetect® *Cis*-Reporting Systems were purchased from Stratagene (La Jolla, CA). All plasmids were expanded in DH5α E.coli and isolated using Qiagen endotoxin-free plasmid Giga-prep kits (Qiagen, Valencia, CA). Other materials included Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) from GIBCO, Invitrogen Corp. (Carlsband, CA), tris(hydroxymethyl)aminomethane (Tris)–Glycine from Bio-Rad (Hercules, CA), polyvinylidene fluoride (PVDF) membranes from Millipore (Billerica, MA), PhosphoPlus® IκB-alpha (Ser32) Antibody Kit from Cell Signaling Technology, Inc. (Beverly, MA) and Puregene Genomic DNA Purification kit from Gentra Systems (Minneapolis, MN).

2.2. Animals

The 6- to 8-week-old female Balb/c and C57Bl/6 mice (Charles River, Wilmington, MA) and 6- to 8-week-old male athymic nu/nu mice (NIH, Bethesda, MD) were used in this study. The animals were kept in groups of five and fed ad libitum. The animal experiments were approved by the University of Nebraska Medical Center Institutional Animal Care and Use Committee (IACUC Protocol # 00-116-10).

2.3. Formulation of plasmid DNA with P85

P85 (lot # WPAU-549B) was obtained from BASF Corp. (Mount Olive, New Jersey). The 10% w/v P85 stock solution was prepared by dissolving P85 in sterile phosphate buffer saline (PBS), and subsequently sterilized using 0.22 μm polysulfone membrane filter (Nalgene, Rochester, NY). For *i.m.* injections, the DNA and P85 stock solution were added into Hanks balanced salt solution (HBSS) buffer and gently mixed to get the required final concentrations of DNA and P85 per 50 μL of dosing solution for each injection, pH7.4. The formulation was used immediately for *i.m.* injection but it was stable for several months if stored at 4 °C. The formulations of DNA with SP1017 were prepared the same way using a 2.25% w/v SP1017 solution (lot # CTM-657) provided by Supratek Pharma Inc. (Montreal, Quebec).

2.4. Ethidium Bromide (EB) assay

The gWiz™ Luc plasmid (0.1 mg/ml) was mixed with EB (2 molecules per 5 bp) and then titrated with 10% P85 solution in PBS pH 7.4 or PBS alone. EB fluorescence was recorded using a Shimadzu P5000 spectrofluorimeter at excitation and emission wavelengths of 520 and 582 nm, respectively. Sam-