Supplementary Information

Synergistic effects on gene delivery – Co-formulation of small disulfide-linked dendritic polycations with lipofectamine 2000™

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Experimental

Materials

Cystamine dihydrochloride (**G0-SS-G0**) and 25 kDa poly(ethyleneimine) were purchased from Aldrich and used as supplied. Polyamines based on L-lysine (**G1-SS-G1** and **G2-SS-G2**) were synthesized using methods previously reported by us and fully characterized; all data were in agreement with those previously published.[1] Lipofectamine 2000^{TM} and Opti-mem buffer were purchased from Invitrogen and used as supplied.

The MDA-MB-231 human breast carcinoma cell line was purchased from the American Type Culture Collection, and the C2C12 murine myoblast cell line was a gift from Prof. Stephen Kaufman (University of Illinois at Urbana Champaign, IL). All cell lines were maintained according to their respective ATCC protocols at 37°C and 5% CO_2 but were adapted from fetal bovine serum to heat-inactivated horse serum. The 5.3 kilobase pair expression vector, pGL3 (Promega, Madison, WI), containing the luciferase gene driven by the SV40 promoter and enhancer, was grown in DH5α E. Coli (Gibco BRL, Rockville, MD) and purified using a commercial plasmid purification kit (Bio-Rad, Hercules, CA). Plasmids were further purified by ethanol precipitation; the ratio of absorbances at 260 and 280 nm was 1.8 or greater.

Methods

(i) Gel Retardation Studies

Appropriate amounts of polyamine and DNA plasmid solutions in 150 mM NaCl (pH 7.3, 20 mM PIPES as buffer) were added to a further 10 μL of 150 mM NaCl (pH 7.3, 20 mM PIPES as buffer) to achieve the desired polyamine/DNA ratio. The resultant complexes were incubated at 4°C for 15 min. Then, 3 μL Blue/Orange 6X loading dye (Promega, Madison, WI) was added, and the mixtures were incubated at 4°C for a further 5 min. After this time, 15 μL was electrophoresed on a 0.75% agarose gel (70 V, 1 h). DNA was visualized with ethidium bromide (Bio-Rad, Hercules, CA). Samples were run in duplicate.

(ii) Cell Formation and Transfection

Lipofectamine 2000™/DNA complexes were prepared at room temperature in Opti-mem buffer according to the manufacturers guidelines, and the desired polyamine/Lipofectamine 2000™/DNA ratio was achieved by addition of a solution of polyamine in Opti-mem buffer, mixing by vortex, and incubation at 4°C for 15 min. Cells were cultured in standard growth medium (DMEM supplemented according to ATCC protocols with 10% horse serum and 1% penicillin streptomycin) and plated in 12-well plates at 1 x 105 cells per well 24 h before transfection. Immediately before transfection, the growth medium was replaced with serum-free medium, and 50 μL of complex (1 μg of plasmid per well) was added to each well. Transfection medium was replaced with growth medium 4 h after transfection. Luciferase expression was quantified 20 h later using a Promega luciferase assay system ($n \geq 6$) according to the manufacturer's protocol. Luciferase activity was measured in relative light units (RLU) using a Lumat LB9507 luminometer (Berthold, GMBH, Germany) and converted to luciferase concentration by comparison with recombinant luciferase standards (Promega). Results were normalized to total cell protein as determined using a Bio-Rad protein assay kit.

(iii) Cytotoxicity Determination

Cytotoxicity was characterized as a decrease in metabolic activity using the XTT assay.[2] Cells were placed in 96-well plates at an initial density of 5000 cells per well in 100 μL of growth medium for 24 h, after which time the growth medium was replaced with fresh serum-free medium. At this point a solution of: polyamine alone (5 μg of polyamine per well), polyamine/DNA complexes (1 μg of DNA and 5 μg of polyamine per well), or polyamine/Lipofectamine 2000™/DNA (effective transfection conditions): 40 μg of polyamine, 0.1 μg of DNA and 0.25 μg of Lipofectamine 2000™ in 10 μL of Opti-mem buffer was added to each well. Cells were incubated for 4 h, and the medium was replaced with complete growth medium for 16 h. Fresh XTT (1 mg/mL) and the coenzyme Q0 (80 mg/mL) stock were prepared each day in PBS (0.5 μg/μL XTT and 0.04 μg/μL coenzyme Q0), and 25 μL aliquots were added to each well. The samples were incubated for a further 4 h at

37°C, and the absorbance was read at 450 nm relative to blank well prepared without cells (n = 16).

References

1 (a) C. S. Love, V. Chechik, D. K. Smith and C. Brennan, *J. Mater. Chem.* 2004, **14**, 919-923. (b) C. S. Love, A. R. Hirst, V. Chechik, D. K. Smith, I. Ashworth, C. Brennan, *Langmuir* 2004, **20**, 6580-6585. (c) C. S. Love, V. Chechik, D. K. Smith, K. Wilson, I. Ashworth and C. Brennan, *Chem. Commun.* 2005, 1971-1973.

2 M. G. Stevens and S. Olsen, *J. Immunol. Methods* 1993, **157**, 225-231.