

Neomycin improves cationic lipid-mediated transfection of DNA in human cells

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Abstract—Delivery of oligonucleotides has been a major impediment in the development of nucleic acid based drugs. In this report, we show that neomycin, an aminoglycoside antibiotic, when combined with a cationic lipid preparation such as DOTAP, enhances transfection efficiency of both reporter plasmids and oligonucleotides and results in a significant increase in transgene expression. The results described here open a new lead in ongoing efforts for oligonucleotide delivery.

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DNA delivery, especially by non-viral means (i.e., transfection), has become an important research tool for understanding gene structure, regulation, and function.¹ Efficient transfection of foreign DNA in cells, however, remains a difficult objective to achieve. It is important to develop systems that are highly efficient in DNA delivery and transgene expression and, at the same time, can be safely applied to basic and clinical research settings. Currently, cationic lipid-based systems are probably the most commonly used methods of DNA delivery, are relatively non-toxic and have been used occasionally in humans. However, transfection efficiency with these systems remains low and less efficient when compared to viral based DNA delivery system. The overall efficiency of transfection in the case of transgene expression vectors depends on both the efficiency of DNA delivery into cells (uptake) and the efficiency of transgene expression, which is determined by the fraction of vector molecules that enter the nucleus and undergo transcription. It is desirable that delivery systems improve both these processes to effectively carry vector DNA into nuclei. A similar problem exists with oligonucleotides, such as triplex forming oligonucleotides (TFOs), that need to enter the cell nucleus in order to interact with their intracellular target, the chromosomal DNA. Thus, unlike antisense oligonucleotides, intranuclear delivery of

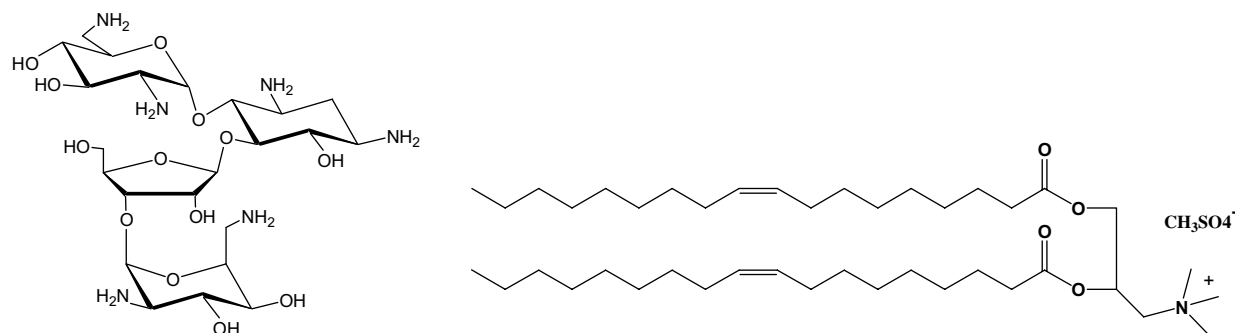
expression vectors and TFOs is an essential and often limiting step for their biological activity.

In the present study, we evaluated the effect of the aminoglycoside antibiotic neomycin on cationic lipid-mediated delivery of plasmid DNA and oligonucleotides in cells. We have previously shown that neomycin can stabilize multiplex nucleic acid structures, such as DNA triplex, RNA triplex, and other hybrid forms.^{2–6} An important question in the possible therapeutic application of these findings was the effect neomycin may have on DNA transfection. Our results indicate that neomycin, when combined with a cationic lipid preparation such as DOTAP (Scheme 1), enhances transfection efficiency of both reporter plasmids and oligonucleotides and results in a significant increase in transgene expression. The enhancing effect of neomycin seems to be mediated by increased uptake across the plasma membrane, although other mechanisms could favorably contribute to the phenomenon.

We determined the effects of neomycin in combination with the cationic lipid preparation DOTAP on the uptake of luciferase reporter plasmids. DU145 prostate cancer cells were transfected with the reporter plasmid pRL-SV40 using DOTAP in combination with increasing concentrations of neomycin. After 24 h, cells were lysed and assayed for luciferase activity. The efficiency of transfection as assessed by the luciferase assay increased as the concentration of neomycin increased from 0.5 to 5 μ M (Fig. 1A). Next, we transfected DU145 with

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Scheme 1. Structure of neomycin (left) and DOTAP (right).

the pGL3-Ets2 and pRL-SV40 reporter vectors in the presence of DOTAP, neomycin, or both DOTAP and neomycin. We observed again an increase in luciferase activity from both reporter vectors when the plasmids were transfected with the combination of DOTAP and 5 μ M neomycin compared to DOTAP alone (Fig. 1B). At 10 μ M of neomycin the effect on luciferase activity was still evident (>2-fold increase) but somewhat reduced compared to the lower concentration of neomycin, suggesting that an optimal ratio between DOTAP, neomycin, and DNA might be needed to achieve optimal results. A likely explanation of these data is that the increased activity of the luciferase reporters in the presence of neomycin might be due to increased cellular uptake of plasmid DNA. It is interesting to note that we did not observe any effect on luciferase reporter activity when neomycin was added to the medium after the transfection (data not shown), indicating that neomycin must be present in the transfection mix in order to improve transfection efficiency.

We examined further the effects of neomycin on transfection efficiency using another reporter system, a plasmid expressing green fluorescent protein, the pEGFP reporter vector. DU145 cells were transfected with the pEGFP plasmid in the presence of DOTAP, neomycin, or both DOTAP and neomycin. After 24 h cells were collected and analyzed for EGFP expression by flow cytometry to examine both fluorescence intensity and percentage of EGFP-positive cells. There was a 5-fold increase in the number of EGFP-positive cells when DOTAP was combined with neomycin compared to

DOTAP alone (Fig. 2B and C). Neomycin alone marginally affected efficiency of DNA uptake. The mean fluorescence intensity was slightly higher in cells transfected with DOTAP and neomycin compared to DOTAP alone (mean fluorescent intensity: 31.9, 27.8, and 17.2 in cells transfected with DOTAP and neomycin, DOTAP alone, and without DOTAP, respectively).

Neomycin appeared to positively affect transfection efficiency of plasmid DNA mediated by DOTAP. This effect could be mediated by an increase in intracellular uptake and/or by an enhanced release of DNA from liposome complex into the cytoplasm. To determine whether neomycin had a similar effect on the uptake of oligonucleotides, we evaluated transfection efficiency

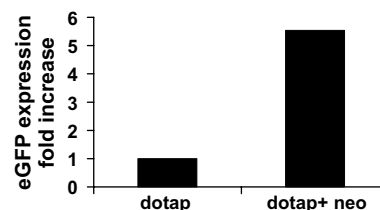


Figure 2. Increased transfection efficiency of an EGFP expressing plasmid in the presence of neomycin. DU145 cells were transfected with the pEGFP plasmid (4 μ g) using DOTAP, neomycin (5 μ M), or both DOTAP and neomycin. Cells were analyzed by flow cytometry after 24 h. Numbers in the figure represent the percentage of EGFP-positive cells (fluorescence intensity >101) in the samples. See [supplementary data](#) for a plot of the % increase in the number of EGFP-positive cells relative to DOTAP or neomycin alone.

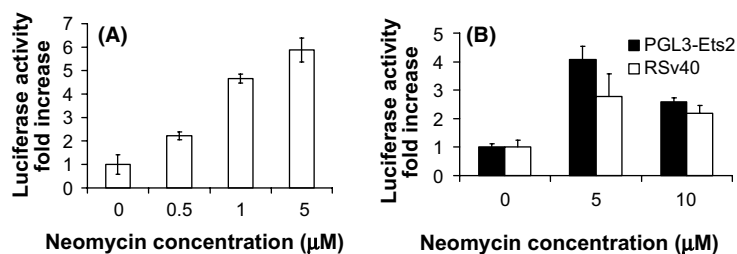


Figure 1. Neomycin enhances cellular uptake of luciferase reporter plasmids. (A) Cells were transfected with the pRL-SV40 reporter vector using DOTAP and the indicated concentrations of neomycin. An oligonucleotide was added to the transfection mix as carrier to adjust the total amount of DNA to 200 ng/well. Luciferase activity was determined 24 h after transfection. (B) DU145 cells were transfected with the pGL3-Ets2 and pRL-SV40 vectors (200 ng/well) using DOTAP or DOTAP combined with neomycin. Luciferase activity was measured 24 h later using the dual-luciferase assay system.

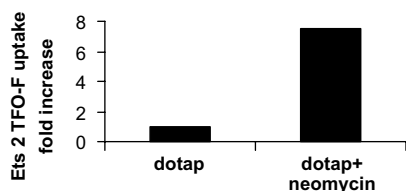


Figure 3. Neomycin increases uptake of fluorescein-labeled oligonucleotide. DU145 cells were transfected with a fluorescein-labeled phosphorothioate TFO (125 nM) in the presence of DOTAP, neomycin (5 μ M), or a combination of DOTAP and neomycin. See [supplementary data](#) for dot plots of fluorescence signals and % increases relative to cells incubated with DOTAP or neomycin alone.

of a fluorescein-labeled oligonucleotide in the presence or absence of neomycin. For the experiments shown in [Figure 3](#), a phosphorothioate oligonucleotide directed to the Ets2 gene promoter (Ets2-TFO) was conjugated at the 5' with fluorescein.⁷ Cells were transfected with the fluorescein-labeled TFO (F-TFO) for 6 h and then incubated for 24 h in fresh medium before the analysis by flow cytometry. At the concentration used in the experiment only about 7% of fluorescein-positive cells were detected when transfected with DOTAP alone. The percentage of fluorescein-positive cells increased to about 50% when the oligonucleotide was delivered using the combination of DOTAP and neomycin. Therefore, neomycin enhanced uptake of the F-TFO by approximately 7-fold. Mean fluorescence intensity was also increased in cells transfected with DOTAP and neomycin compared to cells transfected with DOTAP alone (mean fluorescent intensity: 24.1, 19.4, and 17.6 in cells transfected with DOTAP and neomycin, DOTAP alone, and without DOTAP, respectively). Neomycin alone did not affect the oligonucleotide uptake (2.4% fluorescein-positive cells).

Thus, neomycin improves cationic lipid-mediated transfection efficiency of reporter plasmids and intracellular uptake of oligonucleotides. Our observations suggest that the increased reporter activity observed using the combination of DOTAP and neomycin is mediated by increased intracellular delivery of DNA. It is possible that the adjuvant effect of neomycin is also mediated by additional mechanisms, such as facilitated release from lysosome and nuclear uptake.⁸ Collectively, our data indicate that neomycin enhances DNA and oligo-

nucleotide transfection efficiency mediated by cationic lipid reagents like DOTAP. This effect is mediated at least in part by increased intracellular uptake. The precise mechanism for this phenomenon is currently under investigation and will be reported in due course.

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Supplementary data

Experimental/solution conditions and plots of the % increase in the number of EGFP-positive cells/fluorescent ODNs relative to DOTAP or neomycin alone. Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.bmcl.2005.04.038](https://doi.org/10.1016/j.bmcl.2005.04.038).

References and notes

- (a) Luo, D.; Saltzman, M. *Nat. Biotechnol.* **2000**, *18*, 33; (b) Vijayanathan, V.; Thomas, T.; Thomas, T. *J. Biochemistry* **2002**, *41*, 14085.
- (a) Arya, D. P.; Micovic, L.; Charles, I.; Coffee, R. L., Jr.; Willis, B.; Xue, L. *J. Am. Chem. Soc.* **2003**, *125*, 3733; (b) Arya, D. P.; Xue, L.; Tennant, P. *J. Am. Chem. Soc.* **2003**, *125*, 8070; (c) Xue, L.; Charles, I.; Arya, D. P. *Chem. Commun.* **2002**, 70.
- (a) Arya, D. P.; Xue, L.; Willis, B. *J. Am. Chem. Soc.* **2003**, *125*, 10148; (b) Arya, D. P. DNA Binders. In *Topics in Current Chemistry*; Chaires, J. B., Waring, M., Eds.; 2005; Vol. 253, p 149.
- Arya, D. P.; Coffee, R. L., Jr.; Willis, B.; Abramovitch, A. I. *J. Am. Chem. Soc.* **2001**, *123*, 5385.
- Arya, D. P.; Coffee, R. L., Jr.; Charles, I. *J. Am. Chem. Soc.* **2001**, *123*, 11093.
- Arya, D. P.; Coffee, R. L., Jr. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1897.
- Carbone, G. M.; McGuffie, E. M.; Collier, A.; Catapano, C. V. *Nucleic Acids Res.* **2003**, *31*, 833.
- Lin, A. J.; Slack, N. L.; Ahmad, A.; George, C. X.; Samuel, C. E.; Safinya, C. R. *Biophys. J.* **2003**, *84*, 3307.