Non-Toxic Endogenous Nucleotides for Endosomal Disruption and Enhanced Gene Transfection

H. Cho¹, Y.H. Bae²,³, and H.C. Kang¹*

¹Department of Pharmacy, The Catholic University of Korea, Bucheon, Gyeonggi-do 420-743, Republic of Korea; ²Department of Pharmaceutics and Pharmaceutical Chemistry, The University of Utah, Salt Lake City, Utah 84112, USA; ³Utah-Inha Drug Delivery Systems and Advanced Therapeutics Research Center, Incheon 406-840, Republic of Korea

myone89@naver.com; *Corresponding author: hckang@catholic.ac.kr

ABSTRACT SUMMARY

To escape non-viral vector systems from endolysosomal compartments, the carriers should contain endosomolytic moieties. This study investigated endosomolytic functions of nucleotides such as adenosine triphosphate (ATP) and guanosine triphosphate (GTP) and their transfection enhancement in polymeric gene delivery (Figure 1). Nucleotides exhibited proton buffering capacities and/or hemolytic activities in endolysosomal pH ranges due to their secondary phosphate groups. When the nucleotides were physically loaded into polymer-based gene complexes, the resulting nanocomplexes reached gene expression levels of up to 220-fold higher than their nucleotide-free counterparts with no noticeable cytotoxicity observed in either cancer or non-cancer cells.

INTRODUCTION

Most nano-sized carriers developed for intracellular drug delivery use various endocytic pathways for cellular internalization.¹ Following endocytosis, labile therapeutic payloads, such as proteins and genetic materials, must be released from endolysosomal compartments. To promote this release, endolysosomolytic agents that have pH-dependent conformational transformations, protonable moieties, or both, have often been physically or chemically incorporated into the nanocarriers to disrupt the endolysosomal membrane.

The secondary phosphate groups of nucleotides have a pKₐ of 6.1~6.5,² which falls within the pH range of early endosomes. Thus, this study has examined the use of non-toxic endogenous nucleotides as endosomolytic agents in polymeric gene delivery.

EXPERIMENTAL METHODS

To evaluate endosomolytic functions of nucleotides, pH-dependent proton buffering capacities and hemolytic activity were investigated.

After loading nucleotides (NT) into polymer/pDNA complexes, polymer/NT-pDNA complexes were examined in terms of size, zeta-potential, NT loading contents, cytotoxicity, transfection efficiency, and so on.

RESULTS AND DISCUSSION

NT showed much less cytotoxicity than well-known endosomolytic agents such as chloroquine and bPEI₂₅kDa. All tested NTs
exhibited high proton buffering capacity over a pH range of 5.1~7.4 (Figure 2). In a hemolysis study, nucleotides showed different pH-dependent hemolytic patterns; ATP and ADP disrupted 50~80% of red blood cells below pH 6.0 although they exhibited no hemolytic activity at pH values above 6.5 (Figure 3).

Figure 3. pH-dependent hemolytic activities of nucleotides

As the NT increased in dose from ≤ 0.5 to 1 nmol in bPEI25kDa/NT-pDNA complexes, their sizes increased from approximately 100 nm to 400-500 nm. Doses of NT above 1 nmol caused the complexes to return to approximately 200 nm. Also, the strongly positive zeta potential of polycation/NT-pDNA complexes decreased with the addition of NT.

With ≤ NT1nmol, bPEI25kDa/NT-pDNA trapped more than 90% of available ATP or GTP. However, NT’s loading efficiency declined above NT2nmol, reaching approximately 40~50% for bPEI25kDa/NT8nmol-pDNA. In addition, the NT content was 30~33 wt% in bPEI25kDa/NT2nmol-pDNA and reached 45~54 wt% in bPEI25kDa/NT8nmol-pDNA.

At 2 d post-transfection, bPEI25kDa/NT-pLuc complexes showed negligible cytotoxicity in HepG2 and HeLa cells. However, their highest transfection efficiencies were represented with the addition of NT4nmol for ATP and NT2nmol for GTP. ATP containing complexes exhibited up to 80-fold and 60-fold higher transfection efficiencies in HepG2 and HeLa cancer cells, respectively, whereas bPEI25kDa/GTP-pLuc complexes exhibited up to 25-fold and 16-fold higher transfection efficiencies in HepG2 and HeLa cells, respectively. (Figure 4) Furthermore, in transfection studies using non-cancerous HEK293 cells, bPEI25kDa/ATP-pLuc (NT1nmol and NT2nmol) complexes showed approximately 150~220-fold higher transfection efficiencies than NT-free bPEI25kDa/pLuc complexes.

Figure 4. Transfection efficiencies of bPEI25kDa/NT-pLuc complexes in HepG2 and HeLa cells.

Intracellular localization studies of bPEI25kDa/ATP2nmol-pDNA complexes showed a higher distribution within the cytoplasm than that achieved with bPEI25kDa/pDNA complexes, suggesting that NT facilitates endosomal escape of bPEI25kDa/ATP2nmol-pDNA complexes.

CONCLUSION

In conclusion, nucleotides exhibited proton-buffering capacities and/or hemolytic activities in endolysosomal pH ranges. NT-containing polyplexes showed greater enhancement of transfection with negligible cytotoxicity.

REFERENCES


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