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Research Article

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# GLACTOSYLATED LIPOSOME-POLYETHYLENEIMINE-DNA NANOCOMPLEXES AS A NON-VIRAL VECTOR

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## ABSTRACT

In the present study, combinations of galactosylated liposomes, linear polyethylenimine (PEI) and plasmid DNA (pDNA) were prepared in order to improve gene expression of pDNA. The prepared nanocomplexes were characterized for their size and zeta potential. Luciferase reporter gene was used for determination of transfection activity in Neuro2A cells. Mean particle size of prepared vectors was ranged from 119 to 199 nm whereas the zeta potential varied from 30-50 mV. Transfection efficiency of selected of non-viral vectors was higher than that of PEI 10 KDa. The transfection activity of these polycationic liposomes was reduced in higher carrier to plasmid (C/P) ratio. Moreover, non-viral vectors described in this study showed low cytotoxicity. The results show that PEI in combination with galactosylated liposomes can improve the transfection efficiency in Neuro2A cells.

**Keywords:** Gene delivery, Glactosylated liposomes, Nanocomplexes, Polyethyleneimine.

## INTRODUCTION

Application of viral vectors has restricted due to safety concerns and immunogenicity. Different research groups are working to develop efficient and safe alternative for viral vectors. It has been demonstrated that many cationic polymers and lipid-based DNA delivery systems increase DNA uptake by cells. Probably, lipid-based systems are the most commonly used methods for gene transfer but they still have important drawbacks. The major limitation of this approach is low transfection efficiency.[1-3]

Recently, liposome-polycation DNA complexes (lipopolyplexes) have developed to improve gene expression.[4-8] In these structures, the polycation such protamine or polyethyleneimine can condense DNA into a compact structure, which improve stability of DNA and help gene transfer. In the study of Schafer *et al*, certain lipopolyplexes established by liposome and PEI complexes, showed improved biological properties over PEI complexes.[6] Preparation of PEI lipopolyplexes with 1,2-di-O-octadecenyl-3-trimethylammonium propane (DOTMA) and DNA was reported by Matsumoto *et al*. Increased gene expression in HepG2 cells and animal model was reported.[7] Liposome-protamine-DNA (LPD) nanocomplexes were prepared by Huang group. These structures are virus like structures with a core of protamine-DNA complex and a lipid membrane coat. These vectors were much more stable and also more efficient in transfecting cells *in-vitro*.[8]

In the present study, ternary complexes (lipopolyplexes) composed of linear PEIs (2.5, 25 and 250 kDa) and galctosylated liposomal formulations same as LPD structure were prepared and characterized. Three galactosylated lipids were used in the formulation of liposomes. Also, the biological activity of prepared nanostructures was test in Neuro2A cells.

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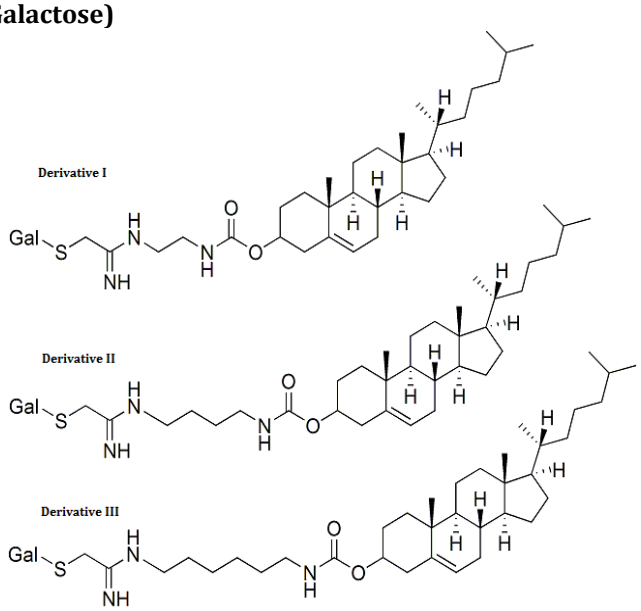
## MATERIALS AND METHODS

### Synthesis of galactosylated lipids

Firstly, Cholesteryl oligoamines were synthesized according to our previous study.[9] Then, Cholesteryl oligoamines and trifluoroacetic acid were reacted in pyridine with 2-Imino-2-methoxyethyl-1-thiogalactoside. The synthesized derivatives were then characterized by FT-IR, TLC and $^1$H-NMR (Figure **1**).

**Figure 1. Structure of galactosylated lipids (Gal: Galactose)**



**Preparation of cationic liposomes and lipopolyplexes**

Liposomal formulation composed of DOTAP, cholesterol and galactosylated lipid at molar ratio of 2:1:1 was prepared by solvent evaporation method.[10] To prepare nano-sized liposomes, the thermobarrel Extruder (Northernlipids, Vancouver, Canada) was used. Liposomes were extruded repeatedly through 400 and 100 nm polycarbonate membranes. To prepare lipopolyplexes, first different amounts of liposomes and PEI were mixed together then pDNA (Plasmid DNA encoding *Renilla* luciferase) was added to the premixing liposome-polycation solution at different polycation to pDNA weight ratios ranging from 0.5:1 to 3:1.

**Zeta potential and size analysis of lipopolyplexes**

The surface charge and particle size of lipopolyplexes were analyzed using a Malvern Zetasizer nano ZS (Malvern, Worcestershire, UK).

***In-vitro* transfection experiments**

Neuro2A murine neuroblastoma cells (ATCC CCL-131), were grown in DMEM supplemented with 10% FBS, streptomycin at 100 µg/ml and penicillin at 100 U/ml. Cells were seeded at a density of $1\times10^4$cells/well in 96-well plates one day prior to transfection experiments. Nanocomplexes were further incubated for 20 min at room temperature and added to the cells. Transfection was performed in complete medium for 4 hours. The medium was replaced with a fresh complete medium and gene expression was assayed 24 hours post-transfection.[9]

**Cytotoxicity of lipopolyplexes**

Cells were seeded in 96-well plates and treated after 24 h with the same amounts of lipopolyplex used for transfection experiment. After 4 h, medium was replaced fresh culture medium. Metabolic activity of each well was determined using a methylthiazoletetrazolium (MTT)/thiazolyl blue assay after 24 h. cell viability was expressed as a percent relative to untreated control cells.[9]

**Statistical Analysis**

One-way ANOVA statistical test was used to assess the significance of the differences among various groups. In the case of a significant F value, multiple comparison Tukey test was used to compare the means of different groups. Results with $p<0.05$ were considered to be statistically significant.

## RESULTS AND DISCUSSION

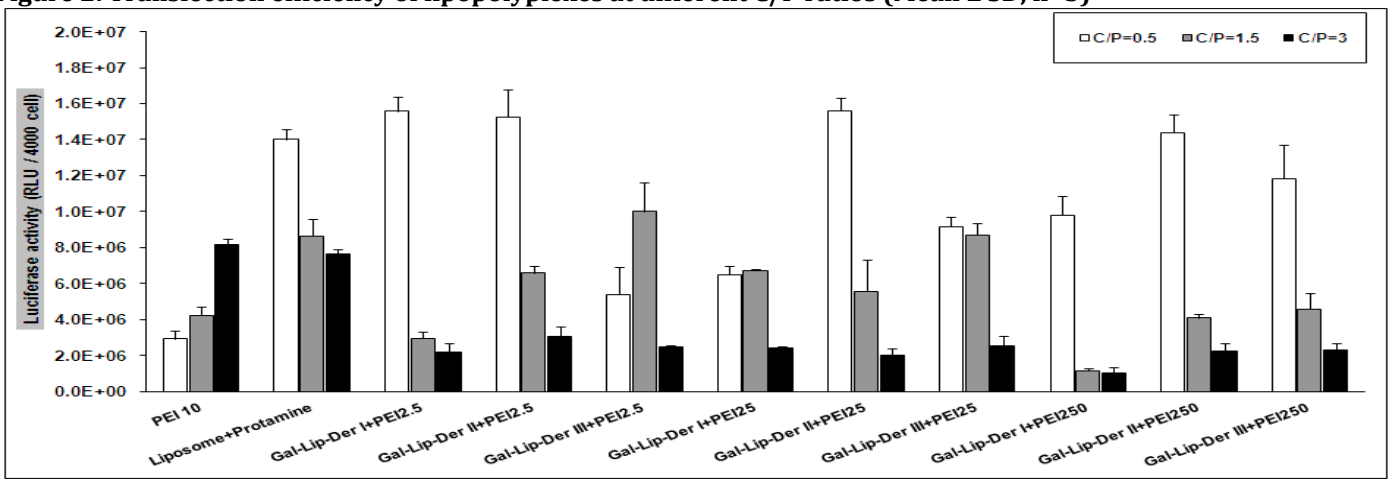
Table **1** shows the mean size and zeta potential of different vectors prepared in the present study. All vectors showed positive zeta potential. Mean size of lipopolyplexes was ranged from 119 to 199 nm. Vectors containing derivative II had higher mean size compared to other vectors. Molecular weight of PEI did not have any significant effect on the final size of carrier.

**Table 1. Size and zeta potential of lipopolyplexes at different C/P ratios (Mean ± SD, n=3).**

| Vector | Z-average (nm) | Zeta Potential (mV) |
|---|---|---|
| DOTAP:Chol:Der I | 126.0±3.6 | 38.8±2.6 |
| DOTAP:Chol:Der II | 149.3±2.0 | 46.8±1.2 |
| DOTAP:Chol:Der III | 116.0±1.0 | 50.2±2.5 |
| DOTAP:Chol:Der I+PEI 2.5 | 141.3±1.8 | 35.1±1.2 |
| DOTAP:Chol:Der II+PEI 2.5 | 168.3±2.0 | 41.9±1.8 |
| DOTAP:Chol:Der III+PEI 2.5 | 119.4±2.3 | 45.1±1.2 |
| DOTAP:Chol:Der I+PEI 250 | 155.2±4.7 | 30.3±1.5 |
| DOTAP:Chol:Der II+PEI 250 | 199.4±8.7 | 36.7±1.4 |
| DOTAP:Chol:Der III+PEI 250 | 146.2±18.2 | 39.1±2.2 |

In this study, lipopolyplex-mediated gene transfer into Neuro2A cells was investigated. As it was presented in Figure **2**, better transfection activity was achieved in the combination of PEI 2.5 KDa and liposomes containing derivative II.

**Figure 2. Transfection efficiency of lipopolyplexes at different C/P ratios (Mean ± SD, n=3)**




Furthermore, interesting point in transfection results was remarkably reduction in transfection efficiency by increasing in C/P ratio. The best gene expression was obtained at C/P ratio of 0.5. Lipopolyplexes containing PEI 2.5 and derivative II had highest gene expression at C/P ratio of 1.5. At C/P ratio of 3, there was no significant ($p>0.05$) differences in luciferase activity between PEI 2.5, 25 and 25 kDa. In some cases, the result of gene expression of prepared vector was better than that of PEI 10KDa and LPD.

All polycationic liposomes induced low cytotoxicity (Figure **3**). Cell viability of these vectors was between 80% and 95% of control. The lipopolyplexes were more toxic by increasing in C/P ratio but no significant differences in cell viability was found ($p>0.05$). Moreover, different molecular weights of linear PEI did not have an effect on the toxicity of lipopolyplexes in transfected cells.(Figure **3**)

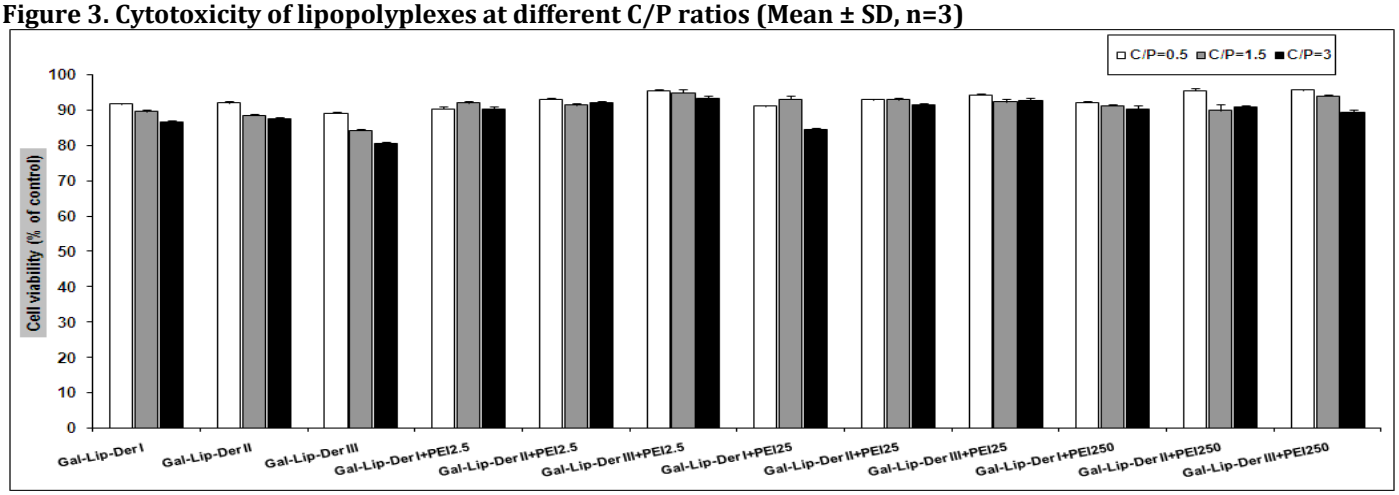
In the present study, novel gene delivery systems composed of galactosylated lipososmes, PEI and pDNA were prepared. Transfection efficiency of selected of these non-viral vectors was higher than that of PEI 10 KDa. The designed gene carriers had negligible cytotoxicity to Neuro2A cells.

It has been shown that overcoming of several biological barriers such as plasma membrane, endosome membrane and nuclear envelope is prerequisite for gene expression.[11]

Lipopolyplexes as a second generation synthetic gene delivery systems are comprised of a mixture of cationic polymer and liposome. In these structures, both PEI and

liposome are able to condense DNA efficiently, so nano-sized complexes will achieve. On the other hand, PEI destabilizes the endosomal membrane due to proton sponge effect, releasing DNA in the cytoplasm.[12]

**Figure 3. Cytotoxicity of lipopolyplexes at different C/P ratios (Mean ± SD, n=3)**




However, high molecular weight PEIs have been used successfully for efficient gene transfer and low molecular weight PEIs cannot be effective to deliver the genes into cells.[4,13] Combination of PEIs and liposomes can enhance transfection activity. In the present study, this synergistic effect was observed by using both low and high molecular weight PEIs. In the study of Lampela et al, high synergism was achieved with PEIs (700 and 2000 Da) and DOTAP liposomes in the C6 cells.[5] They showed that the observed synergism depends on the cell line and the transfection carrier. Although the transfection activity of PEIs is related to the molecular weight and structure (linear or branch) but size of PEIs in the designed vectors in the present study did not have any remarkable effect on transfection efficiency. It has been suggested that two independent mechanisms (improvement of cellular uptake of complexes by liposomes and enhanced transfer from lysosome to cytoplasm by PEI) are determinant in this synergism.[14] It has been shown that galactose is the promising targeting ligand to hepatocytes (liver parenchymal cells) because these cells possess a large number of asialoglycoprotein receptors that recognize the galactose units on the galactosylated carriers.[15,16] As galactosylated LPD showed promising effect in Neuro2A cells. In next step, the biological activity of these carriers should be evaluated in HepG2 cells.

## CONCLUSION

The results indicate that the designed systems are promising carriers for oligonucleotides delivery in Neuro2A cells.

## ACKNOWLEDGEMENT

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