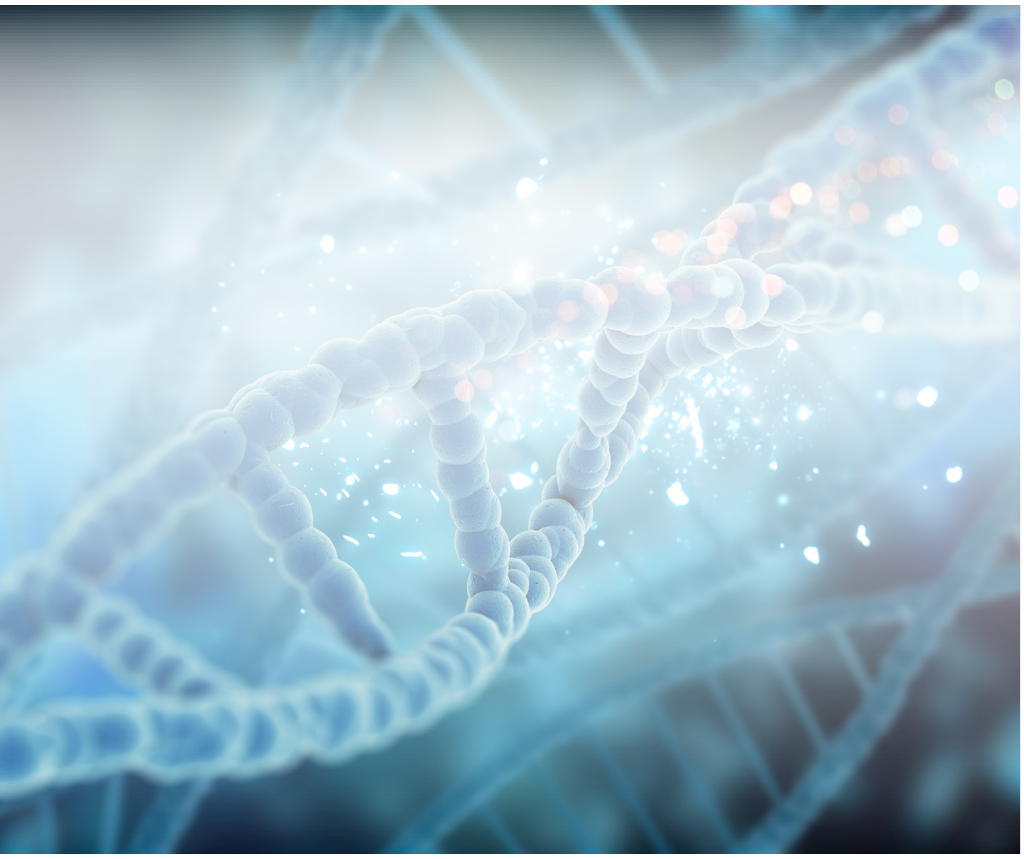

Challenge

Investigation and biodistribution of a gene carrier

Solution

Ex vivo fluorescent imaging using the iBox Explorer² Live Cell Imaging Microscope

Lipodendriplexes Mediated Enhanced Gene Delivery Using the iBox Explorer²

Introduction

Gene therapy has become a novel practice in treating illnesses like cancer. This method of treatment focuses on altering the genetic makeup to change the way a cell functions. Gene therapy is demonstrated by inserting a transgene, a non-native segment of DNA from one organism, into the DNA of another. However, clinical success of effective gene therapy is mainly hampered by the insufficiency of safe and efficient internalization of a transgene to the targeted cellular site. Therefore, the development of a safe and efficient nanocarrier system is one of the fundamental challenges to transfer the therapeutic genes to the diseased cells. Polyamidoamine (PAMAM) dendrimer has been used as an efficient non-viral gene vector (dendriplexes) but the toxicity and unusual biodistribution induced by the terminal amino groups (–NH2) limit its in vivo applications. Hence, a lipid modification (lipodendriplexes) with the PAMAM based gene carrier was studied to investigate their behavior.

In this investigation, dendriplexes and lipodendriplexes labeled with green fluorescent protein-expressing plasmids were compared and analyzed ex vivo. The UVP iBox Explorer² Live Cell Imaging Microscope allows researchers to detect fluorescent markers in small animals from the macro-level to micro-level – from the whole animal to individual cell, subcutaneously and within the body cavity of mice.

Materials and Methods

Samples and reagents

- Mice
- 1,2-Dipalmitoylphosphatidylcholine (DPPC)
- Cholesterol
- PAMAM dendrimer
- Chloroform
- Methanol
- HEPES-5% glucose buffer (HBG)
- 100 nm polycarbonate membrane
- pCMV-GFP (kanamycin-resistant & green fluorescent protein-expressing plasmids)

Samples preparation

PLiposomes were prepared by the thin-film hydration method, as described in previous literature. For the preparation of liposomal formulation, lipids of DPPC and cholesterol at the molar ratio of 85:15 were dissolved in chloroform:methanol (2:1, v/v) mixture. The organic phase was removed by a rotary evaporator to get a thin lipid film. The lipid film was then hydrated with pH 7.4 HBG buffer and sonicated for 10 min at 45 ˚C to get multilamellar liposomes. The liposomal suspension was then slowly extruded through a 100 nm polycarbonate membrane, using a pre-heated mini extruder, to get a unilamellar liposomal suspension.

For dendriplexes, pDNA and PAMAM were dissolved in HBG buffer and were mixed by vigorous pipetting (equal volumes at the N/P ratio 12/1; the ratio of terminal amino groups in the PAMAM dendrimer to the phosphate groups of the nucleic acid) followed by incubation at room temperature for 30 min. Lipodendriplexes were formed by incubating dendriplexes with a fine liposomal dispersion in HBG buffer (liposome to PAMAM mass ratio 0.5/1) for 1 h (equal volumes).

For biodistribution analysis, 7-8 week old mice were divided into groups and i.v. injected with the complexes (dendriplexes and lipodendriplexes) containing pCMV-GFP (10 µg) by tail vein. The mice were sacrificed 24 h post injection and the vital organs were collected for ex vivo imaging.

Imaging

The UVP iBox Explorer² small animal imaging system, having customized wavelength filters for green fluorescence (ex.455 nm–495 nm, em.503 nm–523 nm) was used for the measurement of the GFP expression in vital organs. The results were analyzed by using VisionWorks® software.

Results and Discussion

The precise biodistribution of GFP labeled DNA complexes following i.v. administration was assessed in vital organs, using a fluorescence iBox Explorer2 imaging system. The images of the dissected organs were taken 24 h after the administration of the complexes to detect the fluorescence signals of GFP expression. In the case of dendriplexes the highest signals were detected in the liver followed by lungs, kidneys, heart, and then in the spleen. The liposome modification with dendriplexes significantly increased the fluorescence intensity in all organs, except the spleen, in comparison to naked dendriplexes. The fluorescence signals in the liver appeared to be highest with lipodendriplexes treatment, followed by lungs, kidneys, heart, and then in the spleen. (Fig 1 a & b).

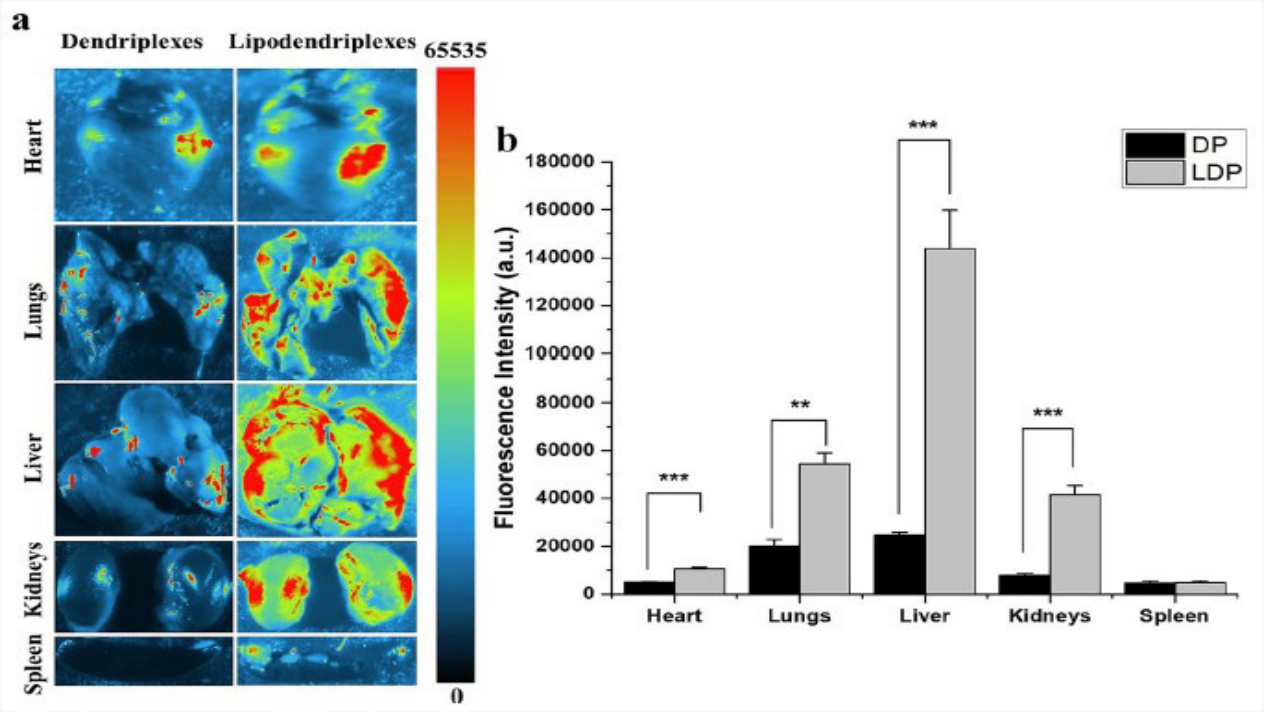

Figure 1 Ex vivo fluorescence analysis of vital organs i.v. administration of the complexes containing 10 µg of pCMV-GFP (dendriplexes and lipodendriplexes of DPPC:CH-PAMAM; liposome to PAMAM dendrimer mass ratio 0.5/1 with N/P ratio 12/1). The mice were sacrificed 24 h after the administration of the complexes. The organs were collected carefully and washed with normal saline to remove any blood traces. (a) Ex vivo fluorescence images using UVP iBox Explorer² small animal imaging system using wavelength filters for green fluorescence (excitement 455 nm–495 nm, emission 503 nm–523 nm). Fluorescence intensity is demonstrated by a color scale bar (red depicting maximum fluorescence intensity; 65535, while dark blue is minimum fluorescence intensity; 0) and (b) quantitative biodistribution (fluorescence intensity a.u.) of pCMV-GFP labeled DNA complexes in vital organs. [Black graph bar represents dendriplexes (DP) and grey bar graph represents lipodendriplexes (LDP)]. Values are represented as mean ± SD (n = 3) and statistical significances are indicated as **p < 0.01, ***p < 0.001.

Conclusion

An efficient and safe gene delivery system was created by incorporating a PAMAM based dendriplexes system with an optimized liposomal formulation of DPPC:CH (85:15). The results revealed that the incorporation of liposome with naked dendriplexes has essentially increased the cellular uptake of the complexes, which was confirmed by ex vivo fluorescence imaging of the dissected organs. From the findings, it could be concluded that the development of such a non-viral nanocarrier system could be considered for an efficient gene transfection with a better safety profile, in both in vitro and in vivo delivery systems.

References

[1] Tariq, I., Ali, M.Y., Sohail, M.F. et al. Lipodendriplexes mediated enhanced gene delivery: a cellular to pre-clinical investigation. Sci Rep 10, 21446 (2020). https://doi.org/10.1038/s41598-020-78123-6

Headquarters
Analytik Jena US LLC
2066 West 11th Street
91786 Upland · USA

Phone +1 909 946 3197
Fax +1 909 946 3597

info@us.analytik-jena.com
www.analytik-jena.us

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