Application Note

Fluorescence *in Vivo* Imaging of labeled Nanoparticles in Tumour bearing Mice with NightOWL LB 983

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Abstract

Systemic delivery systems are needed for therapeutic application to organs that are inaccessible by percutaneous administration.

In the present study, the main objective was to develop a stable and non-toxic synthetic formulation that can deliver foreign genetic material to target cells, such as tumour cells.

To visualize the tumour accumulation of the complexes, *in vivo* fluorescence imaging was performed. The developed formulation allowed passive targeting without causing any side effects and seems to be an excellent candidate for an efficient *in vivo* transfection.

Introduction

For the treatment of unreachable organs and disseminated or metastatic cancer, it is now essential to develop stable and specific intravenous forms of therapy.

However, systemic targeting remains a real challenge because of complexation with serum proteins and toxicity or clearance by the mononuclear phagocyte system (MPS).

In previous works, lipid nanoparticles were developed leading to really weak complement activation and low macrophage uptake (Heurtault et al., *Pharm Res*, 2002). The formulation of these nanocapsules was adapted to obtain DNA nanoparticles (DNA LNPs).

Thanks to the use of oleic Plurol® instead of Lipoid® in their formulation, the lipid core allowed the entrapment of plasmid DNA molecules via the formation of lipoplexes. Thus, DNA LNPs were small (117 ± 10 nm), suitable for an intravenous injection, but *in vivo* stability and blood half-life remained low.

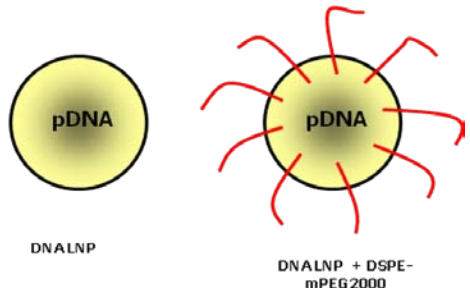

Figure 1: Schematic representation of DNA LNPs

To allow an extended circulation time, and consequently higher tumour selectivity (Maeda et al., *J Control Release*, 2000), authors choosed to modify the surface of our gene delivery systems, by inserting longer PEG (Poly ethylene glycol) chains at the surface of DNA LNPs between the already-existing, dense PEG660 chains. This was carried out through the use of two kinds of

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amphiphilic and flexible polymers (Vonarbourg et al., *Biomaterials*, 2009).
The ability of the different particles to escape complement activation and uptake by THP-1 macrophages was investigated. Then the long circulating properties of these particles *in vivo* after intravenous injection in mice and their tumour accumulation ability by NIR fluorescence imaging system were evaluated. In parallel, blood samples were harvested to measure the hepatotoxic impact of the different formulations before and after injection.

Experimental Procedure

Coated and uncoated DNA nanoparticles were developed by encapsulating DNA into lipid nanoparticles (LNPs) leading to the formation of stable nanocarriers with a size inferior to 130 nm. Amphiphilic and flexible polymer coatings at different concentrations were selected to make DNA LNCs stealthy (Morille et al., *Biomaterials,* 2010*).*
Cytotocixity, hepatoxicity and blood kinetic studies were performed prior *in vivo* imaging. (Morille et al., *Biomaterials,* 2010).
Tumour bearing mice were prepared by injecting subcutaneously a suspension of $1x10^6$ U87MG glioma cell line in 150 mL of Hanks Balanced Saline Solution (HBSS) into the right flank of athymic nude mice (6 weeks old females, 20–24 g)
In order to evaluate the biodistribution of coated DNA LNPs and uncoated DNA LNPs in tumour bearing mice, LNPs were labeled with DiD probe, a near-infrared (NIR) fluorophore. After 21 days, 150 mL of nanoparticles were injected via the tail vein of the mice presenting tumours on their right flank.
Non-invasive fluorescent imaging was then performed 3 h, 5 h, 24 h and 48 h post-injection using the biofluorescence imaging (BFI) system LB 983 NightOWL II equipped with cooled slow scan CCD camera. As DiD fluorescent tag was used to localize the nanoparticles, the 590 nm excitation filter and the 655 nm emission filter were selected. In parallel, the light beam was kept constant for each fluorescent measurement, which was ideal with the ringlight epi illumination. The ringlight was always set at the same height, to ensure that the excitation energy on the sample would be always the same.
Each mouse was anesthetized with a 4% air–isofluran blend. Once laid in the acquisition chamber, the anesthesia of the mice was maintained with a 2% air–isofluran mixture all along the experiment.
With the NightOWL system, the fluorescent acquisition time was 2 seconds, the fluorescent signal was then overlaid on a black-white picture of the mice.
The CCD camera collects light coming out from the skin of the animal without any *a priori* information regarding the deepness of the sources. However, excitation and emission photons employed in these experiments have a mean path before absorption of 1–2 cm, and this property depends on the optical characteristic of tissues themselves. Thus, since the photons can pass up to 2 cm through the animal body, sources located up to 2 cm below the skin can be visualized. However, in order to unambiguously localize the fluorescent dye accumulated in specific anatomical areas, a much more detailed study should be performed.

NightOWL

The NightOWL is an *in vivo* bioluminescence (BLI) and biofluorescence (BFI) imaging system equipped with extreme sensitive CCD cameras (cooled to -80°C).
A variety of options makes easy adaption to different applications possible. Whole animals and even plants can be imaged as well as blots, gels, microplates, cell culture dishes and arrays.

Figure 2: NightOWL II in vivo imaging system

Results

To estimate time dependant excretion profile and tumour accumulation of the coated LNPs exposing the greatest residence time in bloodstream, these suspensions were intravenously injected in the tail vein of tumour bearing mice and compared to non-coated DNA LNPs. Tissue distribution was evaluated thanks to NIR biofluorescence imaging (BFI).

First of all, the early fluorescence signals were much more intense after injection of coated DNA LNPs than after the administration of uncoated particles. When regarding non-covered DNA LNPs, the fluorescence intensity increased in the liver area from 3 h after injection up to 24 h, whereas no accumulation in this anatomical area was observed with DSPE-mPEG2000-coated DNA LNPs at any time (Fig. 3). In parallel, a fluorescence emission was observed 3 h, 5 h, 24 h, and 48 h after DNA LNPs injection on the kidney area, which could therefore let think to an elimination of DNA LNCs via urinary system. At 24 h and 48 h after injection, DSPE-mPEG2000-covered DNA LNPs displayed stronger fluorescence intensity in the tumour and in its vicinity, compared to non-coated DNA nanoparticles.

Conclusion

The DSPE-mPEG2000-coated DNA LNPs developed are able to circulate in the bloodstream without being degraded or captured by the cellular defense mechanisms, and to accumulate in the tumour area. With the NightOWL imaging system biofluorescence tumour monitoring could be performed easily.

One hurdle, the extracellular one, is therefore crossed, but numerous barriers still exists at the cellular level, and efforts have to be made to still improve this vector. Nevertheless, this DNA delivery system seems to be an excellent candidate for an efficient in vivo transfection.

Material

- U87MG glioma cell line (ATCC, Manassas, VA, USA)
- Hanks Balanced Saline Solution (HBSS)
- Athymic nude mice (6 weeks old females, 20–24 g, Charles Rivers, Wilmington, MA)
- DiD probe (Invitrogen)
- NightOWL LB 983 (Berthold Technologies)
- 590/20 nm excitation filter (#37989)
- (Berthold Technologies)
- 655/20 nm emission filter (#51332)
- (Berthold Technologies)
- Isofluorane for anesthesia (Abott)

Literature

Morille M, Montier T, Legras P, Carmoy N, Brodin P, Pitard B, Benoît JP, Passirani C., Long-circulating DNA lipid nanocapsules as new vector for passive tumour targeting, *Biomaterials* 2010; 31(2):321-9.

Heurtault B, Saulnier P, Pech B, Proust JE, Benoit JP. A novel phase inversion-based process for the preparation of lipid nano-carriers. *Pharm Res* 2002; 19(6):875–80

Vonarbourg A, Passirani C, Desigaux L, Allard E, Saulnier P, Lambert O, et al. The encaps-

ulation of DNA molecules within biomimetic lipid nanocapsules. *Biomaterials* 2009; 30 (18):3197–204

Maeda H, Wu J, Sawa T, Matsumura Y, Hori K. Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. *J Control Release* 2000;65(1–2):271–84

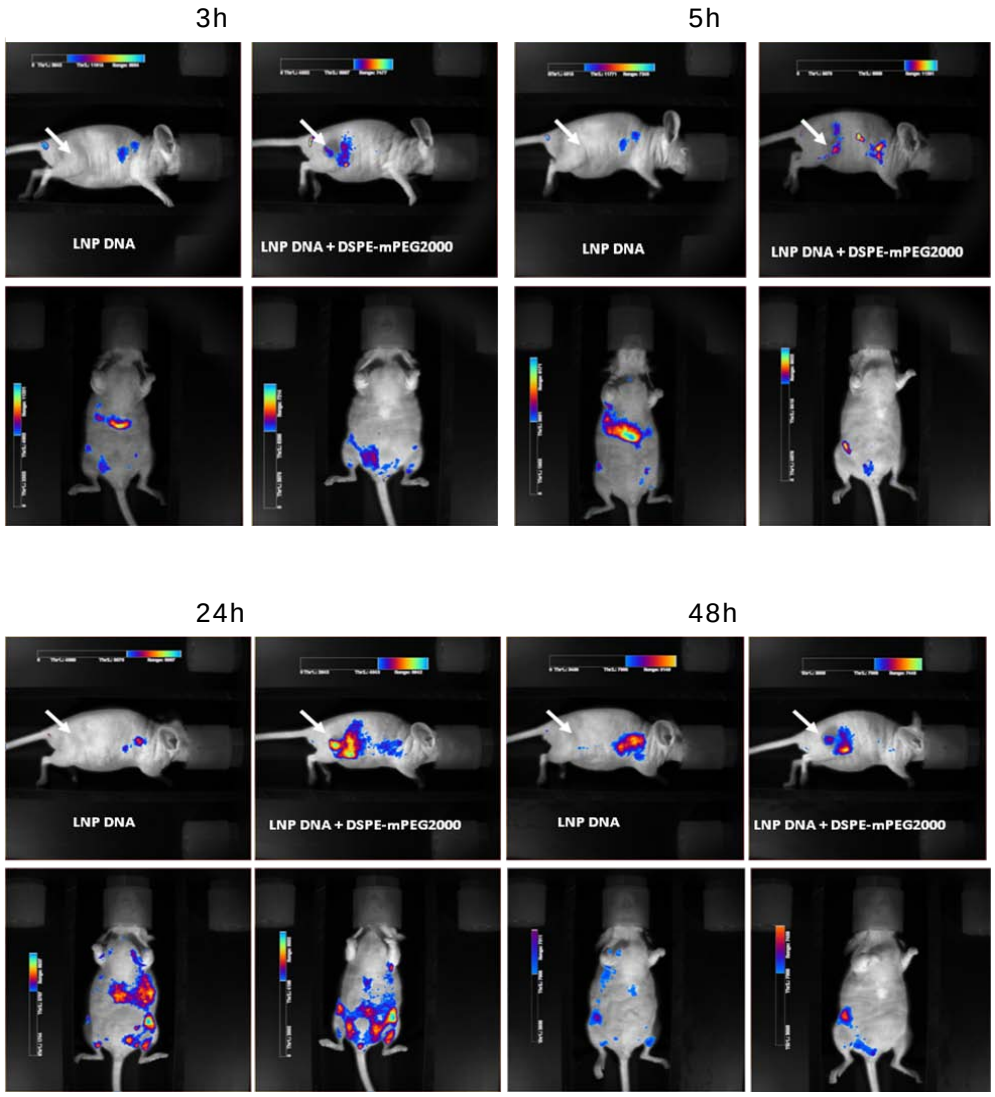

Figure. 3. *In vivo* fluorescence imaging of athymic nude mice bearing U87MG tumors after intravenous injection of DNA LNPs or DSPE-mPEG2000-coated DNA LNPs. Optical images of nude mice with 152 mg/mL tail vein injection of DNA LNPs or DSPE-mPEG2000-coated DNA LNPs (representing 46 mg of pDNA per mice). Coloured bar on the left or upper part of the picture indicates the signal efficient of the fluorescence emission coming out from the animal. The tumour location is specified with a white arrow.