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# Exploring the Spatial Distribution of mRNA-Lipid Nanoparticles in Mouse Whole-Body and Isolated Organs Using MALDI MSI

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## PURPOSE

To investigate the biodistribution and potential toxicity of lipid nanoparticles (LNP1 and LPN2), which are crucial carriers for mRNA-based treatments after administration to male and female mice, by analyzing their distribution in whole-body carcasses and specific organs using MALDI-MSI.

## METHOD(S)

Sectioning performed at 20 µm and 10 µm thicknesses respectively for:

- Whole-body carcasses targeting organs such as the liver, kidney, pancreas, spleen, lung, heart, and testis
- Isolated organs including the liver, spleen, heart, ovary

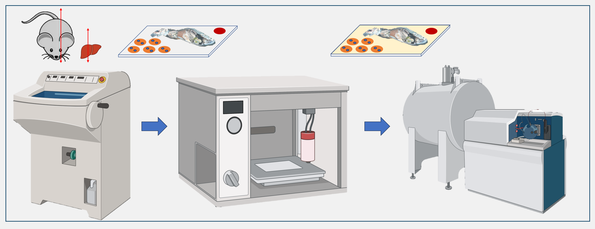
Mounting of the sections on ITO glass slides, drug calibrant deposit on control liver sections and spray of DHB matrix spiked with an internal standard on the slides.

H&E staining of isolated organ sections after analysis and on adjacent sections.

MSI using FlexImaging at 350 µm and 80 µm spatial resolutions for whole-body sections and isolated organs, respectively. Data Analysis software from Bruker Daltonics, alongside Multimaging™ from Aliri France SAS v1.2.6.1. For histological analysis, NDP.view2 by Hamamatsu was utilized.

Drug signal normalized by its internal standard (stable isotopic labeled compound) per pixel of analysis

Correlation of the calibration curve with the tissue signals to measure the concentration of the test substances in µg/g of each histological structure.



## RESULT(S)

### Spatial distribution of LNP1 in mice

LNP1 exhibited a pronounced and enduring presence in both male and female mice, with detectability extending up to 168 hours post-intravenous administration in females (Figure 1) and up to 72 hours in males.

This compound was quickly and broadly distributed among crucial organs such as the spleen, liver, kidney, heart, and intestinal areas with adjacent white fat. Both genders showed particularly high exposure levels in the liver and spleen (Figure 2).

At higher spatial resolution with isolated organs such as the female spleen at T24h, the heterogeneity of LPN1 signal reflected with a higher concentrations in the red pulp compared to the white pulp (Figure 3).

For females, the tissue half-lives were measured between 57 to 88 hours, while in males, these ranged from 30 to 168 hours, with the most minimal clearance rates found in the liver and spleen (Table 1, other organs including kidney, pancreas, lung, heart, and testis not shown).

Note: Results based on n=1 animal per time-point, no inter-individual variability investigated in this study.

### Spatial distribution of LNP2 in mice

In contrast, LNP2 was not detected in female whole-body sections or isolated organs by the 6-hour mark, indicating either limited distribution, rapid metabolism and elimination, or potentially insufficient sensitivity to assess LNP2 exposure.

Figure 1: Spatial distribution of LNP1 in female mouse whole-body sections at T1h, T6h and T168h

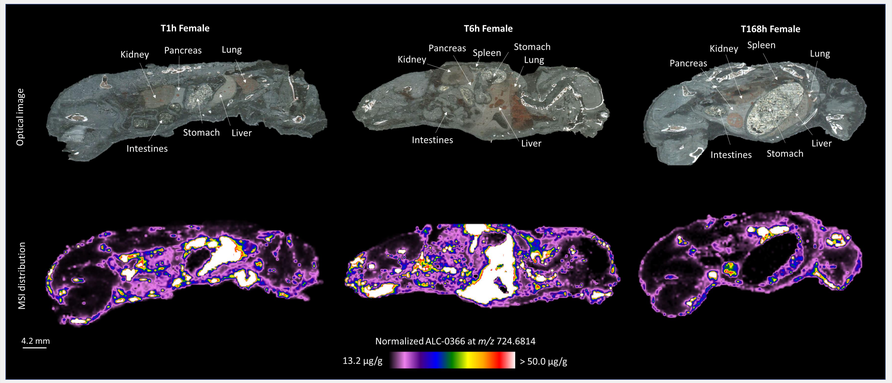



Figure 2: PK profiles of LNP1 in Female carcass organs

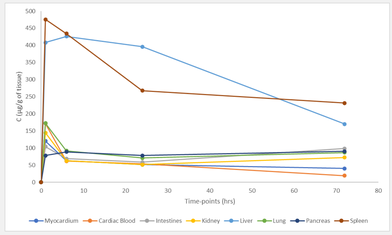



Figure 3: Spatial distribution of LNP1 in female mouse isolated spleen at T24h

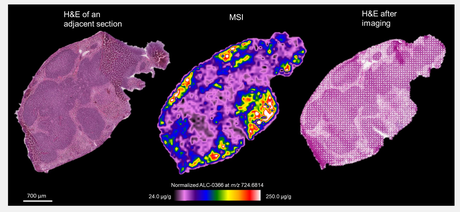



Table 1: PK parameters of LNP1 in Female and Male carcasses-Illustration of Liver and Spleen only)

| | | Liver | | Spleen | |
|---|---|---|---|---|---|
| **Parameter** | **Unit** | **Female** | **Male** | **Female** | **Male** |
| Lambda_z | 1/h | 0.012 | 0.01 | 0.009 | 0.004 |
| t1/2 | h | 57 | 66 | 75 | 168 |
| Tmax | h | 6 | 1 | 1 | 1 |
| Cmax | µg/g | 426 | 414 | 476 | 639 |
| C0 | µg/g | 408 | 452 | 484 | 761 |
| Clast_obs/Cmax | | 0.15 | 0.331 | 0.191 | 0.505 |
| AUC 0-72 | µg/g*h | 34670 | 15860 | 36517 | 23000 |
| AUC 0-inf_obs | µg/g*h | 39911 | 28914 | 46299 | 101120 |
| MRT 0-inf_obs | h | 76.4 | 91.1 | 105.3 | 250.8 |
| Vz/F_obs | g/kg | 10.3 | 16.5 | 11.6 | 12 |
| Cl | g/kg/h | 0.125 | 0.173 | 0.108 | 0.049 |

## CONCLUSION(S)

The study indicates that LNP1 maintains a prolonged presence and wide organ distribution in mice, with significant retention in the liver and spleen. This suggests LNP1's potential as an effective drug delivery system. However, the distinct pharmacokinetic differences between genders underline the necessity for more detailed studies to explore these variances. Meanwhile, the absence of detectable LNP2 in early assessments in females calls for further investigation to assess its delivery efficacy. These results emphasize the importance of thorough preclinical evaluations for lipid nanoparticle-based therapies.

This study pointed out the capability of MALDI-MSI to screen the spatial biodistribution of components of drug delivery systems, to also determine the PK profiles and parameters in a range of targeted organs and other surrounding regions within single whole-body sections requiring minimal sample preparation.

The higher spatial resolution imaging for organs addressed the spatial heterogeneity of the signal correlated with the histological features of the targeted organs for better toxicity assessments.

## REFERENCE

**PK solver**

https://doi.org/10.1016/j.cmpb.2010.01.007