Mapping mRNA–Lipid Nanoparticle Distribution in Mouse Whole Body and Organs by MALDI-MSI

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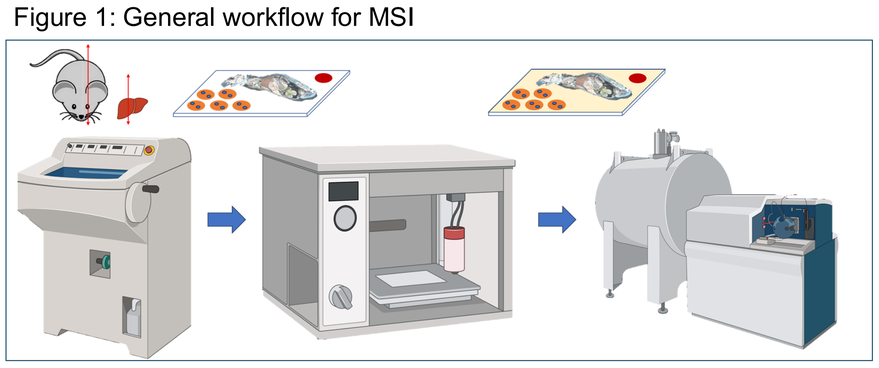
PURPOSE

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, analyzing their distribution in whole-body carcasses and specific organs using MALDI-MSI.

Figure 1: General workflow for MSI



METHODS

- Sectioning was performed at a thickness of 20 µm and 10 µm, for whole-body carcasses and isolated organs, respectively.
- Sections were mounted on ITO glass slides; drug calibrators were spotted on control liver sections and DHB matrix spiked with an internal standard was sprayed on the slides.
- H&E staining of isolated organ sections was performed after analysis and on adjacent sections.
- MSI was performed on a SolariX 7T MALDI FTICR from Bruker Daltonics using FlexImaging at 350 µm and 80 µm spatial resolutions for whole-body sections and isolated organs, respectively.
- Multimaging™ v1.2.6.1 from Aliri France SAS was used for image analysis, drug signal normalization by internal standard and analyte quantification in tissue by correlation of the calibration curve with the tissue signals to determine the concentration of the test substances in µg/g of each histological structure.

RESULTS

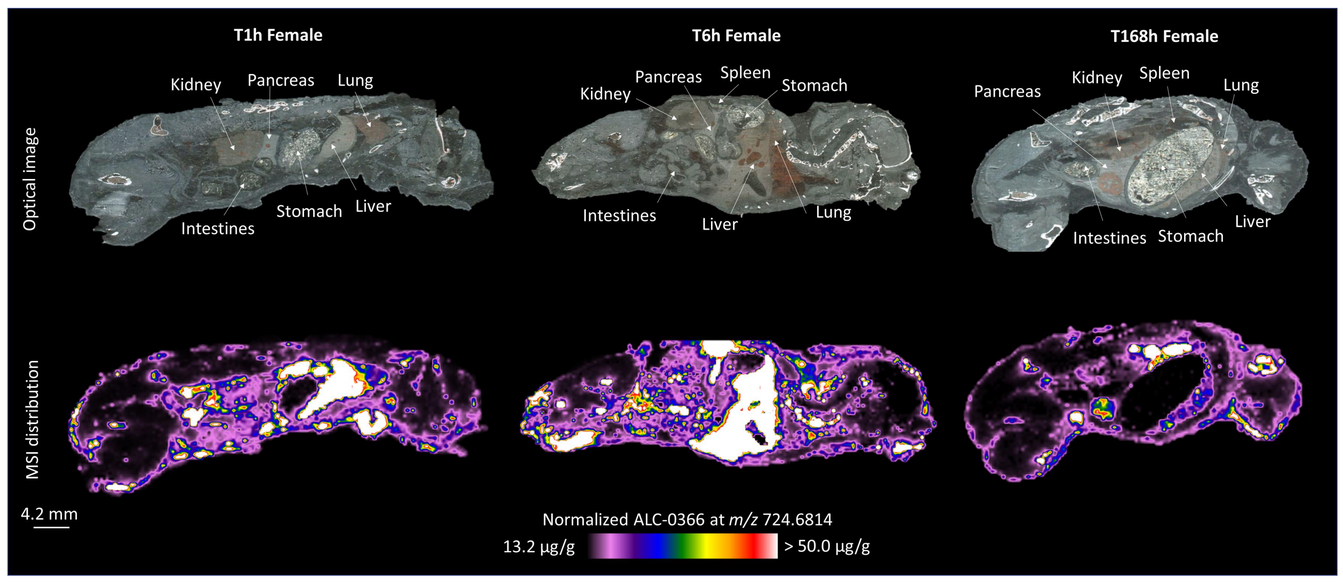
Spatial distribution of LNP1 in mice

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 of the liver and spleen (Figure 2).

At higher spatial resolution, in isolated organs such as the female spleen at T24h, MSI revealed heterogeneous distribution of LPN1 with higher concentrations in the red pulp than in the white pulp (Figure 3).

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



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 not shown).

Figure 2: PK profiles of LNP1 in Female carcass organs

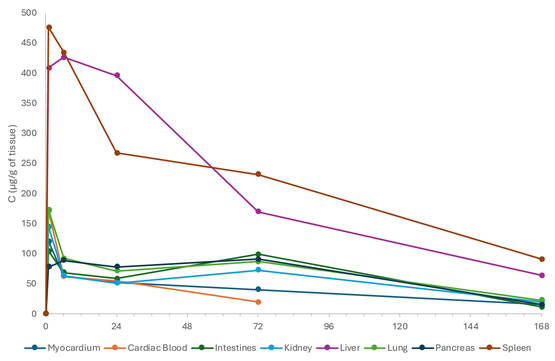


Figure 3: Spatial distribution of LNP1 in female mouse isolated spleen at T24h

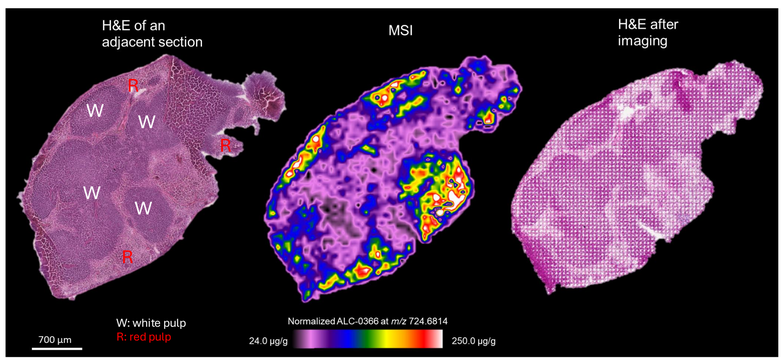


Table 1: PK parameters of LNP1 in Female and Male carcasses- (Illustration of Liver and Spleen only)

Parameter	Unit	Female Liver	Male Liver	Female Spleen	Male Spleen
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

Spatial distribution of LNP2 in mice

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

CONCLUSION

The study indicates that LNP1 maintains a prolonged presence and wide organ distribution in mice, with significant retention in the liver and spleen. 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 points to potential sensitivity limitations of MALDI MSI for this specific class of LNP, calling 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, not only the spatial biodistribution of a drug, but also the components of advanced drug delivery systems such as lipid nanoparticles. It also allowed to determine their PK profiles and PK 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.