Effect of Body Weight and Anti-inflammatory Pre-medications and Concomitant Medications on the Pharmacodynamic Activity and Tolerability of mRNA-Lipid Nanoparticle Formulations in Monkeys

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Abstract

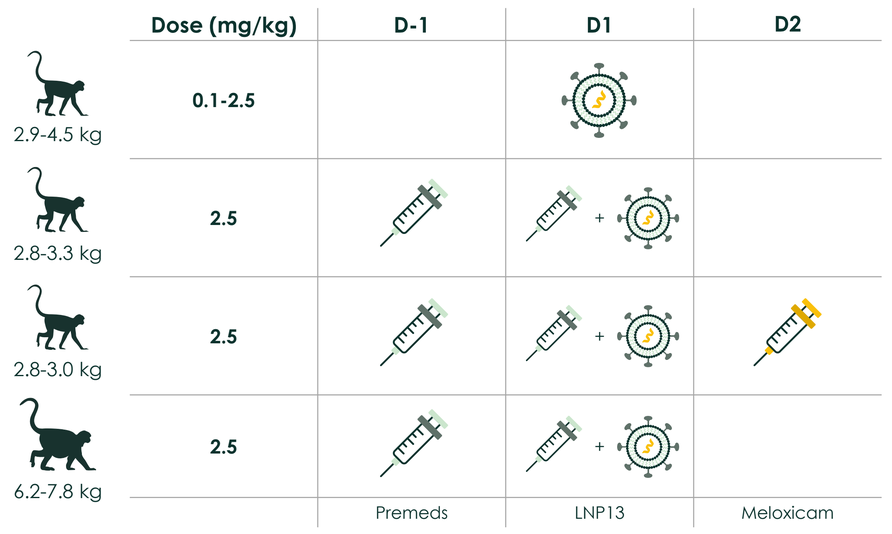
With the ongoing development of lipid nanoparticle (LNP) technology for intravenously (IV) administered therapies, there is increasing need for a detailed understanding of factors influencing LNP activity and tolerability, including pre-medication and concomitant medications used to mitigate expected LNP effects. Additionally, challenges in procuring monkeys in recent years have led to CROs using animals of more varied sources of origin (e.g., Chinese, Cambodian, Vietnamese, Mauritian) and a general trend towards the use of larger animals (>5-6 kg).

To evaluate the impact of animal size, pre-medications (steroid, H1 and H2 blockers) and concomitant medications (meloxicam) on activity and tolerability, monkeys were given LNP containing an mRNA encoding human IgG. LNP were administered via a 1-hour IV infusion to smaller, naïve cynomolgus macaques (2.75-4.5 kg) at various dose levels from 0.1 to 2.5 mg/kg mRNA to fully characterize the activity and tolerability of the mRNA-LNP without pre-medication. At the highest dose (2.5 mg/kg), additional groups of animals received a standard regimen of pre-medications prior to LNP administration, with or without meloxicam (a non-steroidal anti-inflammatory drug commonly used by some CROs). Additionally, a group of larger animals (6.2-7.75 kg) were given mRNA-LNP and pre-medication to further evaluate the impact of animal size on activity and tolerability.

IV administration of mRNA-LNP up to 2.5 mg/kg was tolerated with no mortality/morbidity observed in monkeys and no clinical signs observed at doses ≤1.5 mg/kg. Clinical signs were noted in all groups given 2.5 mg/kg, with the most clinical signs observed in the large (>6 kg) monkeys. At doses of ≥0.75 mg/kg, transient and dose-dependent changes were observed for some clinical pathology parameters (e.g., ALT/AST), peaking 2 days after dosing and exhibiting reversibility 7 days post dose. At 2.5 mg/kg, differences in the magnitude of ALT/AST changes were observed, with the large monkeys and monkeys given no pre-medications exhibiting greater ALT/AST increases compared to smaller monkeys given pre-medications. Changes in platelet (PLT) levels were also observed at the high dose level (2.5 mg/kg), with greater PLT decreases observed in monkeys given meloxicam and in the large monkeys, relative to monkeys given no pre-medication. Dose-dependent increases in plasma IgG expression were observed from 0.1 to 2.5 mg/kg mRNA. The greatest plasma IgG expression was noted in monkeys administered 2.5 mg/kg without pre-medications, and in the large monkeys, whereas smaller monkeys given pre-medications had reduced IgG levels. PK profiles of the aminolipid were similar for all monkeys at the 2.5 mg/kg dose level and were unaffected by pre-medications or concurrent medication.

Overall, these results provide a comprehensive characterization of the activity and tolerability of an mRNA-LNP formulation in monkeys following IV infusion and provide evidence that monkey body weight, pre-medications and meloxicam treatment may impact LNP activity and tolerability and should be carefully considered in study design and execution.

Study Design



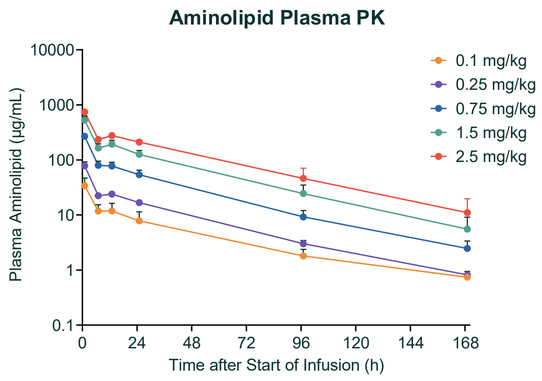
Medication	Class	Dose (mg/kg)	Admin. Route
Dexamethasone	Corticosteroid	1	IM
Famotidine	Antihistamine (H2 blocker)	0.5	IM
Diphenhydramine	Antihistamine (H1 blocker)	5.0	IM
Meloxicam	NSAID	0.2	SQ

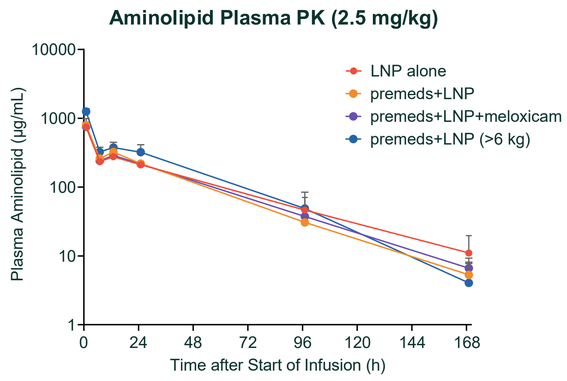
mRNA Dose (mg/kg)	Average Body Weight (kg)	Average mRNA Dose (mg)	Average Lipid Dose (mg)
0.1	3.6	0.36	10
0.25	3.2	0.80	23
0.75	3.6	2.70	76
1.5	3.5	5.25	149
2.5	3.0	7.50	212
2.5	7.2	18	509

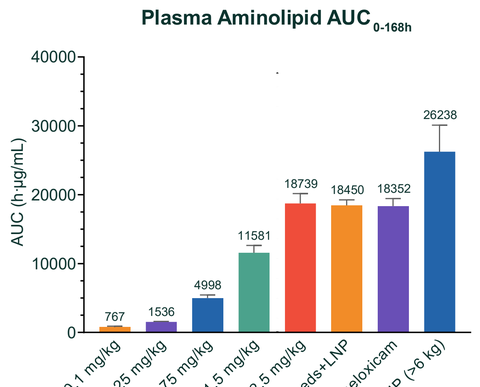
2.4X↑

Study design to evaluate the activity and tolerability of LNP13 (an Acuitas mRNA-LNP formulation) in Chinese cynomolgus macaques. Monkeys were treated with saline or LNP13 administered at 5 dose levels from 0.1-2.5 mg/kg by IV infusion. Additional groups at 2.5 mg/kg were given a standard anti-inflammatory pre-medication regimen consisting of dexamethasone, famotidine, and diphenhydramine with or without meloxicam treatment the day after dosing. Lastly, a group of large monkeys >6 kg was included to assess the effect of body weight on activity and tolerability. The amount of mRNA and total lipid dose administered to the larger animals was 2.4X higher than the smaller monkeys.

Plasma PK is Unaffected by Pre-medications or Meloxicam

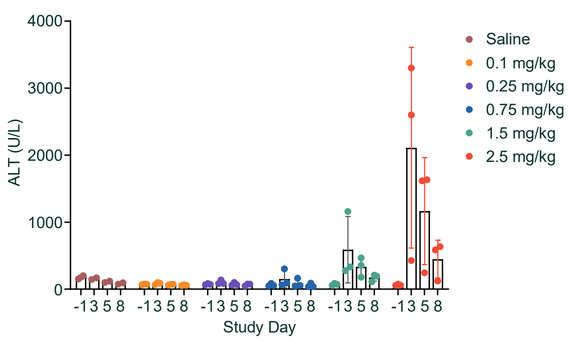


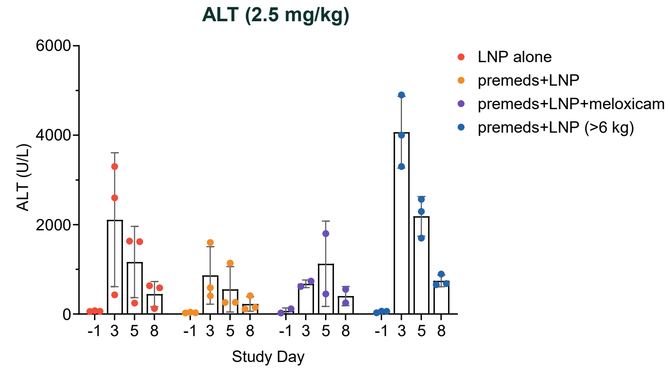


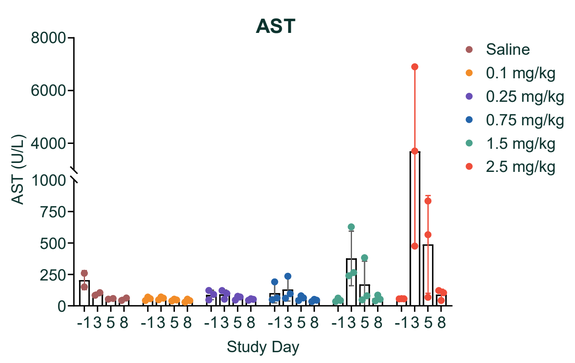


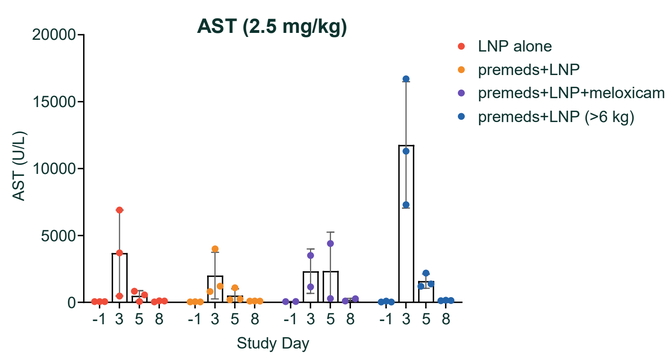
Aminolipid plasma PK profiles. Plasma aminolipid concentration increased in a dose-dependent manner based on the mRNA dose administered. The high dose groups had similar overlapping profiles indicating that treatment with pre-medications or meloxicam did not interfere with LNP plasma exposure. The plasma area under the curve for the large monkeys was 1.4-fold greater than for the smaller monkeys, confirming that the large monkeys had elevated lipid exposure.

Pre-medications Reduce Levels of Liver Transaminases







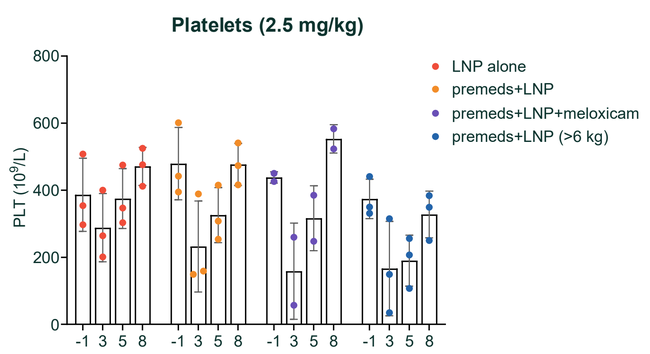


mRNA-LNP Tolerability Assessments

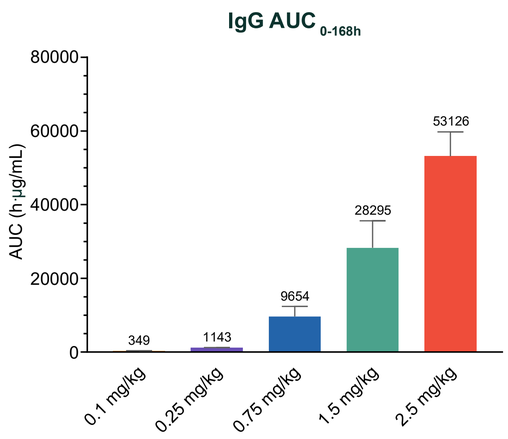
- Minimal increases in ALT or AST and no adverse clinical signs at LNP doses ≤1.5 mg/kg.
- At the high dose, pre-medication helped to reduce the levels of liver enzymes in smaller monkeys. Large animals had the highest ALT/AST elevations despite being given pre-medication.
- Decreased appetite, red spots, and loss of body weight were observed to some degree in all high dose groups. Large monkeys showed the most signs of toxicity and had the highest loss of body weight.
- At 2.5 mg/kg, a transient decrease in platelets was observed that returned to baseline by Day 8. The lowest platelet counts were observed in the group treated with meloxicam and the group of large monkeys.

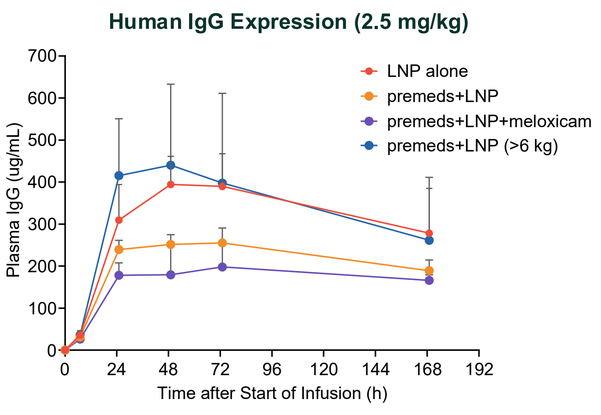
Clinical Observations

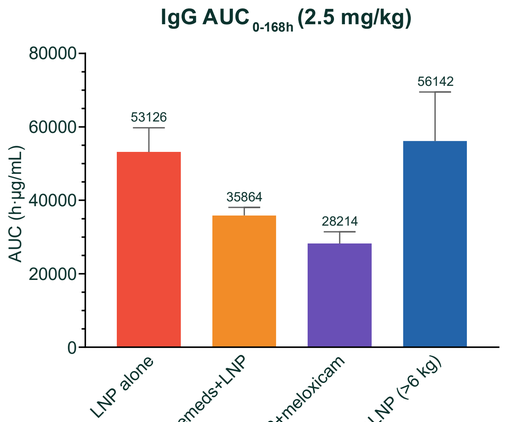
Group	Dose (mg/kg)	Decreased Appetite	Petechiae (rash)	Body Weight Change (D8)
LNP alone	0.1-1.5			-2.2%
LNP alone	2.5	✓(1 of 3)		-1.6%
Pre-meds + LNP	2.5		✓(2 of 3)	-1.7%
Pre-meds + LNP + Meloxicam	2.5		✓(1 of 3)	-6.2%
Pre-meds + LNP (>6kg)	2.5	✓(3 of 3)	✓(3 of 3)	-6.9%



LNP Activity was Influenced by Pre-medications







Human IgG reporter mRNA expression. A dose-dependent increase in the level of plasma IgG was observed from 0.1-2.5 mg/kg doses of mRNA. Use of pre-medication was associated with decreased mRNA activity which was reduced further with addition of meloxicam. Despite increased LNP exposure in large animals, mRNA expression was only slightly increased compared to smaller animals treated with LNP alone.

Summary

1. **LNP13 was well tolerated in the absence of pre-medications. No adverse clinical signs were observed at doses ≤1.5 mg/kg.**
2. **Pre-medications helped to reduce the elevation of liver transaminases in smaller monkeys, but ALT/AST levels remained high in larger monkeys.**
3. **Liver weight may not increase proportionately with body weight, leading to increased liver exposure and elevated toxicity in large monkeys dosed on a mg/kg basis.**
4. **Although pre-medications helped to improve tolerability, they also reduced the level of IgG mRNA expression.**
5. **Caution should be used when considering NSAIDs like meloxicam for supportive care, as clinical pathology (e.g., platelets) and body weight were negatively affected.**
6. **The impact of body weight on tolerability should be considered in NHP study design and in clinical dosing of obese or overweight patient populations.**

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