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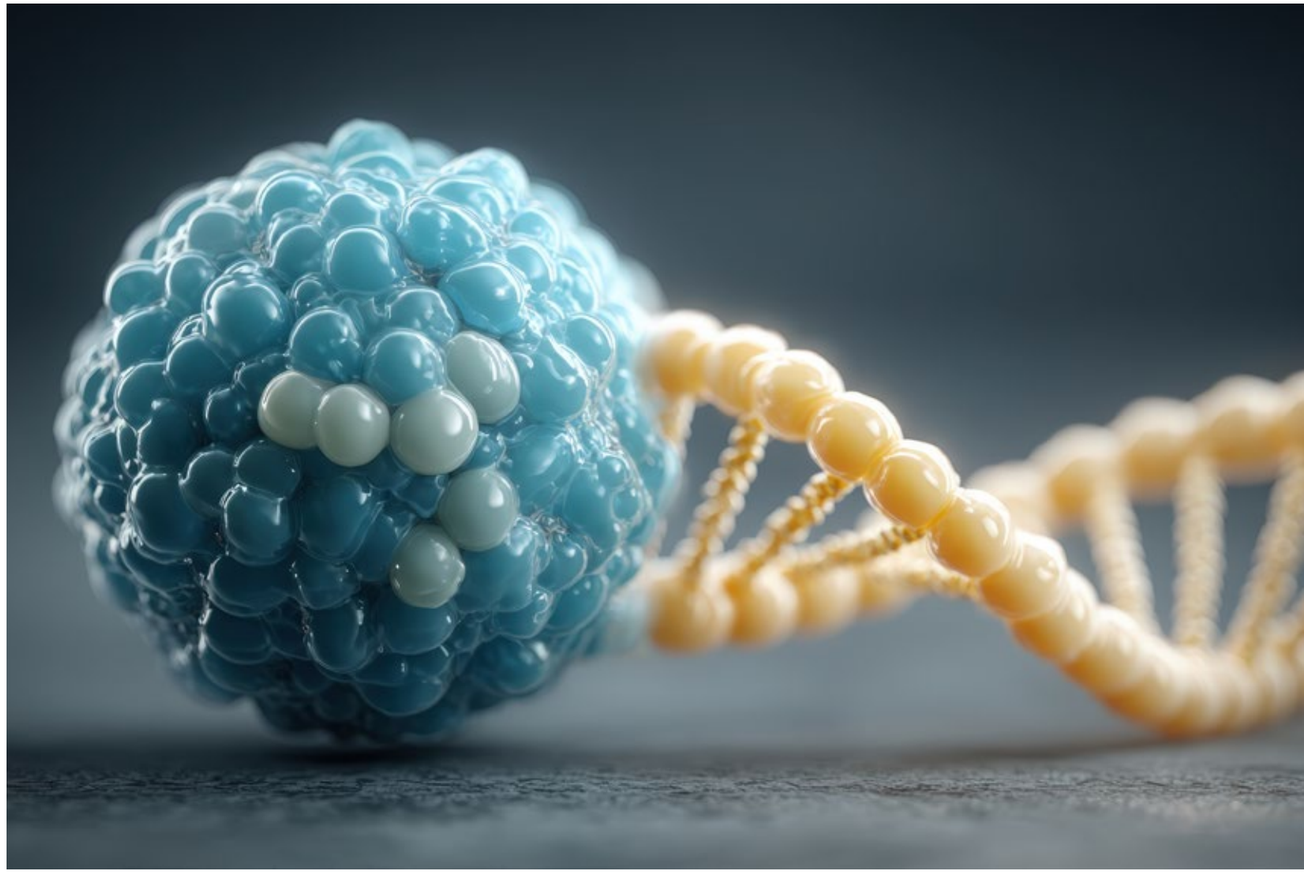

Assessment of Lipid Nanoparticles (LNPs) Caused Side Effects in Preclinical Studies in Non-human Primates

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BACKGROUND & PURPOSE

The application of lipid nanoparticles (LNPs) as a carrier vehicle to protect RNA from rapid degradation and aid intracellular delivery and endosomal escape is a significant improvement in the development of mRNA-based vaccine and novel cancer and/or rare disease therapies. The LNP platform contributes to promoting robust humoral immune response indicating adjuvant activity, and animals receiving the LNP-mRNA combined vaccine often presented with certain type of side effects, such as clinical observations of erythema, swelling, and mass etc., and reactions in various tissue/ organs. The adjuvant activity and reported side effects in animals could stem from the inflammatory properties of LNPs. These side effects may obscure the actual toxicological effects from the test substance and mislead data interpretation in preclinical safety studies. This is especially true in Non-human Primates (NHPs), which are translationally relevant species for assessments of pharmacodynamics, toxicity, and physiological effects of biologics to predict potential adverse effects in humans. Based on these observations, this study aims to present specific parameters or methods for assessment of LNP-associated side effects in preclinical safety evaluation studies with NHPs.

METHODS

To explore and assess LNP-associated side effects in preclinical safety evaluation performed in NHPs, data from 19 preclinical toxicity studies conducted in the past 5 years to evaluate LNP containing substances safety were analyzed. A series of parameters including animal information, clinical signs, body weight, and related-laboratory indexes such as clinical pathology, immunocyte phenotyping, cytokine levels, and tissue/organ response were analyzed.

RESULTS

The data analyses revealed that LNP-associated safety studies were mostly performed in NHPs at dose levels of 1 to 500 μg/kg with ages ranging from 1.2 to 5 years. Clinical signs were not age-related in animals but correlated with body weight gain. However, animal response to LNPs varied based on dose levels and the size of LNPs. Clinical signs mostly related with dose administration site including erythema and/or swelling with incidences ranging from 20.3% to 46.5%, which were observed commonly on the second day after dosing via intramuscular or intradermal inoculations. The issues resolved within 1 to 4 days but might worsen (ulcer and/or mass formation) after repeated dosing. Some animals appeared to have decreased body weight gains (up to 11.2%) over the dosing period due to irritation of the dose administration site, which correlated with the local inflammation caused by the LNPs.

RESULTS

Along with the inflammation, several clinical pathology changes were commonly observed including mild changes in red blood cell (RBC)-related parameters with decreased RBC counts (RBC), hemoglobin (HGB), hematocrit (HCT) and mean corpuscular hemoglobin concentration (MCHC), ranging from 1.3% to 47.6%, increased white blood cell (WBC) counts, decreased lymphocyte (Lymph) counts ranging from about 21.3% to 138.2%. The increase of WBC was characterized with neutrophils (Neut) increase in majority.

Coagulation changes were also commonly observed with increases in APTT and FIB (ranged from 17.3% to 155.1%). In addition, clinical chemistry changes of decreased Albumin (ranged from 13.2% to 32.7%) and A/G (ranged from 21.5% to 48.2%), increased globulin (ranged from 6.1% to 37.2%), were observed among the LNPs-treated animals. Cytokine levels were analyzed as an indicator of tissue responses, although they are not solely attributed to inflammatory reactions. Cytokine changes which correlated to LNP-induced tissue responses mainly included increased IL-6 level (ranged from 53% to 4,034%) observed 1 to 6 hours post dosing, as well as increased C-reactive protein (CRP) ranging from 47% to 704%. However, no changes in immunocyte (lymphocyte) phenotyping and serum complement (C3 and C4) were observed in the animals regardless of dosing routes and dose levels.

Histopathological findings mainly included the dose administration site inflammatory reaction and/or muscle degeneration/necrosis, thymus decreased lymphocyte cellularity, increased tingible body macrophages, liver hepatocyte vacuolation and/or increased ito (hepatic stellate) cells, adrenal gland hemorrhage, necrosis, mineralization, and/or mixed inflammatory cells infiltration, mammary gland atrophy, and prostate gland atrophy, with incidences ranging from 8.3% to 72.3%. These clinical and laboratory changes in this species should be distinguished from toxicological effects of active ingredients in the test materials in preclinical toxicity studies in NHPs and considered as predictive indicators in human clinical trials.

CONCLUSION

The results presented from this review provide reference information for preclinical toxicity assessment studies for drug candidates using LNPs as a delivery system in non-human primates. The data interpretation of preclinical safety studies indicate that NHPs have superior predictive value for human adverse immunological effects. Additionally, NHPs has been demonstrated to be a reliable animal model to provide predictive value for human pharmacodynamics, toxicological impacts, and physiological effects of biologics, the side effects from the LNP delivery platform may obscure the actual toxicological effects from the test substance and result in misleading data.

RESULTS

Table 1 Time-dependent Body Weight Changes versus Predose (%)

LNP Dose Levels (μg/kg)	Number of Animals	Total Number of Animals			
		1	2	3	4
< 50	24	-3.5	0.3	3.6	2.3
51-100	72	-2.8	2.1	2.8	2.9
101-200	48	-4.2	-2.4	-1.8	-3.7
201-300	48	-5.1	-3.1	-2.1	-2.8
301-400	20	-6.7	-5.2	-2.7	-9.7
401-500	12	-5.8	-4.8	-4.6	-11.2

Table 2 Changes of RBC-related Parameters Correlating with Dose Levels (% of decreases compared to predose) after Dosing for 4 weeks

LNP Dose Levels (μg/kg)	Number of Animals	RBC	HGB	HCT	MCHC
< 50	24	2.6	3.2	1.3	0.8
51-100	72	6.2	4.9	1.8	2.1
101-200	48	5.8	3.8	3.2	3.7
201-300	48	11.2	12.7	6.7	9.4
301-400	20	18.9	11.6	13.4	11.7
401-500	12	47.6	16.9	28.7	23.7

Table 3 Changes of WBC-related Parameters Correlating with Dose Levels (% of decreases compared to predose) after Dosing for 4 weeks

LNP Dose Levels (μg/kg)	Number of Animals	WBC	Neut	Lymph	Mono
< 50	24	7.6	16.2	-2.3	-1.4
51-100	72	11.2	17.9	-1.8	0.6
101-200	48	10.8	16.8	-0.4	-1.6
201-300	48	16.2	25.7	3.1	-2.8
301-400	20	23.9	24.6	9.8	-3.1
401-500	12	52.6	29.9	25.1	-2.4

Table 4 Changes of Coagulation Correlating with Dose Levels (% of increases compared to predose) after Dosing for 4 weeks

LNP Dose Levels (μg/kg)	Number of Animals	APTT	PT	FIB
< 50	24	1.3	0.2	7.2
51-100	72	6.7	2.1	18.7
101-200	48	31.2	1.7	34.5
201-300	48	17.4	3.6	75.4
301-400	20	27.3	4.1	155.1
401-500	12	22.5	3.6	82.4

Table 5 Main Changes of Clinical Chemistry Correlating with Dose Levels (% of Changes Compared to Predose) after Dosing for 4 weeks

LNP Dose Levels (μg/kg)	Number of Animals	Alb ↓	A/G ↓	Glb ↑
< 50	24	11.3	21.5	6.1
51-100	72	13.2	23.4	17.4
101-200	48	23.4	26.1	8.7
201-300	48	18.3	31.5	21.5
301-400	20	21.5	29.4	37.2
401-500	12	32.7	48.2	26.8

Table 6 Main Changes of Cytokines Correlating with Dose Levels (% Compared to Predose) after Dosing for 1 to 6 Hours

LNP Dose Levels (μg/kg)	Number of Animals	TGF-β1 ↓	IL-6 ↑	IL-10 ↑	IL-12p40 ↑
< 50	31	1.8	21.0	10.5	6.4
51-100	20	6.3	53.0	11.2	7.5
101-200	13	2.1	168.0	12.3	9.2
201-300	16	5.7	684.0	8.9	5.3
301-400	8	6.8	4034.0	10.7	6.6
401-500	34	2.8	1780.0	23.5	7.2

RESULTS

Figure 1 Summary of Dosing Site Response in Animals after Subcutaneous or Intramuscular Dosing

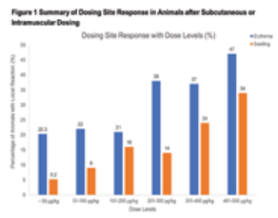


Figure 4 Summary of C-reactive Protein (CRP) Changes in Animals after Dosing for 1 to 6 Hours

Figure 2 Summary of Time-Response in Animals after Dosing (days)

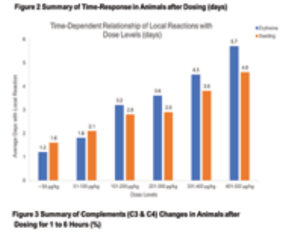


Figure 5 Summary of Affected Organs/Tissues in Animals after Dosing for 4 Weeks

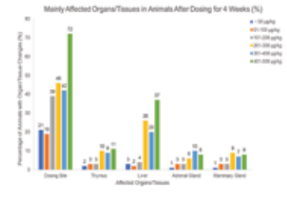
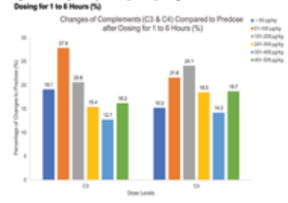


Figure 3 Summary of Complements (C3 & C4) Changes in Animals after Dosing for 1 to 6 Hours (%)



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