Intravenous Administration of Liposome Encapsulated Deferoxamine Efficiently Removes Iron from the Liver and Spleen of Iron Overloaded Mice

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Introduction

Long-term red blood cell transfusions effectively sustains patients who have β-thalassemia, sickle cell anemia, and myelodysplastic syndromes but they also lead to excess iron accumulation in the body. Iron overload is a major cause of morbidity and mortality in transfusion dependent patients. Chelation therapy reverses iron accumulation but marketed chelators have drawbacks such as: long infusions of deferoxamine (DFO, Novartis), large oral tablets with adverse effects (Exjade, Novartis), or twice daily oral dosing (Ferriprox, ApoPharma). These attributes contribute to poor compliance and poor outcomes in iron overload patients. To overcome long infusions and high doses of current therapies we have devised a stable nanoliposome encapsulated DFO (LDFO) for the treatment of iron overload.

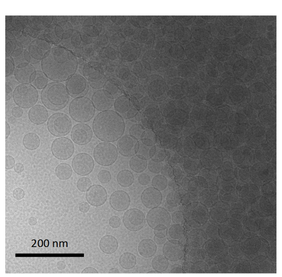
Methods

LDFO composed of saturated soy phosphatidylcholine and cholesterol (3/2 molar ratio) is manufactured using a proprietary remote loading method that provides high encapsulation of DFO in 90 nm diameter liposomes. For pharmacokinetics and bioavailability studies, DFO and lipid concentrations in CF-1 mice plasma and tissues were analyzed by HPLC utilizing an in-house method. For iron removal efficacy studies, CF-1 mice were overloaded with iron dextran and after 10 days washout were treated with 100 mg/kg LDFO or unencapsulated DFO. Animals were sacrificed 5 days post treatment and tissue iron was measured by a ferrozine based spectroscopic assay.

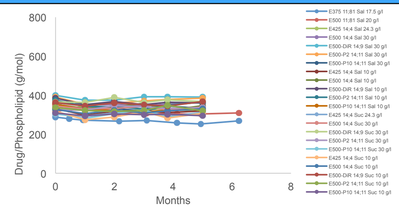
Liposome Encapsulated DFO (LDFO)

Characterization

- Nanoparticles: 90-110 nm in diameter and low polydispersity index (PDI) by dynamic light scattering
- High drug to phospholipid ratio (D/PL): 250-400 g DFO / mole PL
- High drug loading efficiency: >50%
- Cryo-electron microscopy confirms regular morphology of LDFO (102 nm, 0.04 PDI).

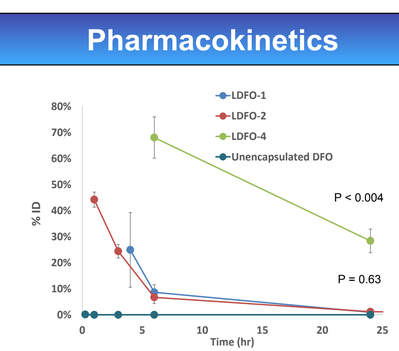


Storage Stability



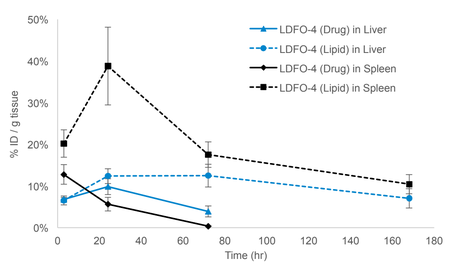
- A variety of LDFO formulations stored at 4 ºC in either HEPES-buffered saline (Sal) or sucrose (Suc) at 10-30 g/l DFO were accessed for formulation-drug retention stability by size-exclusion chromatography and HPLC.
- The majority of the samples tested had high drug retention within 1-3% of the starting D/PL after refrigerated storage over 4 months.
- Active pharmaceutical product degradation studies (<3%) and formulation-size stability both remain relatively unchanged (data not shown).

Pharmacokinetics



- CF-1 mice were administered IV with 100 mg/kg LDFO or unencapsulated DFO.
- LDFO composed of saturated phosphatidylcholine (LDFO-4) exhibits prolonged drug circulation compared to those composed of unsaturated phosphatidylcholine (LDFO-1, LDFO-2) and unencapsulated DFO (not detectable).

Biodistribution



- CF-1 mice were administered IV with 100 mg/kg LDFO or unencapsulated DFO, and tissue were collected and assessed for drug and lipid label DiR.
- LDFO is bioavailable in liver and spleen beyond 72 hours.
  - Instantaneous drug concentrations ≥10% ID / g tissue were detected.
  - Drug clearance from tissues is faster than lipid component.
- Unencapsulated DFO is not detectable (data not shown).

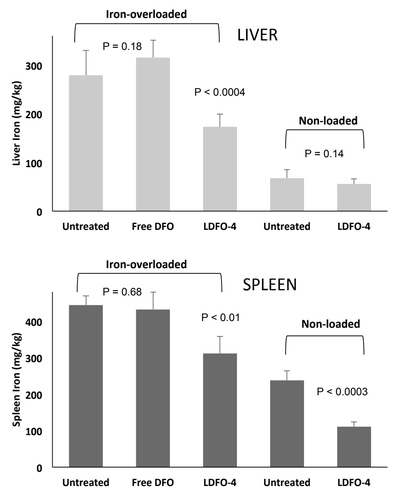
Toxicology

Mouse Weights

			Percent Weight Change		
			First 3 mice	Remaining 3 mice	
Group	Treatment	DFO Dose	Day 1 to Day 3	Day 1 to Day 3	Day 1 to Day 8
1	L-DFO	30 mg/kg IV	0.2 ± 0.8	4.3 ± 3.2	11.5 ± 3.8
2	L-DFO	100 mg/kg IV	4.8 ± 4.3	4.1 ± 1.2	11.5 ± 1.5
3	L-DFO	300 mg/kg IV	3.9 ± 2.6	4.9 ± 1.42	10.1 ± 1.9
4	Saline	-	3.7 ± 3.7	3.6 ± 3.8	16.7 ± 5.7
5	L-DFO	1250 mg/kg IP	5.1 ± 4.8	4.3 ± 14.7	-0.9± 13.1

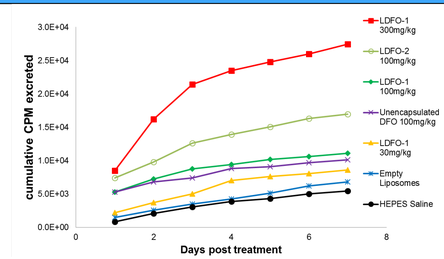
- Single dose safety studies of LDFO formulations composed of unsaturated phosphatidylcholine were performed in CF-1 mice.
- Overall, the test article was generally well tolerated in the IV treated groups at all doses administered, with no animal deaths or effects on body weight, and no abnormal findings or clinical signs of toxicity.
- Serum cholesterol was mildly elevated in a dose-related manner on Day 3 in the IV treatment groups, but returned to baseline levels by Day 8.
- A marked increase in serum cholesterol levels was observed in Group 5 (1250 mg/kg IP) on Day 3 compared to the control group, but reduced by Day 8.
- The no-observed-adverse-effect level (NOAEL) for IV administered LDFO-1 was considered to be 300 mg/kg and 1250 mg/kg when given IP.

Iron Removal



- The iron content of liver (upper panel) and spleen (lower panel) of iron-overloaded CF-1 mice (n=4) five days after treatment with 100 mg/kg unencapsulated DFO or LDFO. The effect of treatment in non-iron overloaded mice (n=4) is also shown.
- The absolute efficiency of LDFO is ~59% on a mole LDFO injected / mole iron removed from the liver and spleen combined.
  - In the liver, 100 mg/kg LDFO eliminates roughly 100 mg/kg liver iron.
  - In the spleen, 100 mg/kg LDFO eliminates roughly 130 mg/kg spleen iron.
- For unencapsulated DFO, there were no statistical significant iron elimination observed in both liver and spleen.
- Iron removal is also observed in non-iron overloaded mice.

Iron Excretion



- Fe-59 urine cumulative excretion from iron-overloaded CF-1 mice after IV dosing with the listed treatment groups (each data point is urine pooled from 7 animals).
- Liposomal delivery of DFO increases iron excretion in urine in a dose dependent manner.

Results

The manufacturing method to prepare LDFO results in a 300 g DFO/mole phospholipid encapsulation ratio. The formulation has greater than 6 months stability at 4 ºC. LDFO is long circulating and the released DFO is bioavailable. At 24 hr post I.V. injection, there is 30% ID DFO in plasma and 10% ID DFO/g in liver whereas unencapsulated DFO is not detectable. Preclinical single dose safety studies in CF-1 mice indicate that LDFO is well tolerated at 300 mg/kg I.V. and 1250 mg/kg I.P. In the iron dextran overload model, LDFO greatly reduces iron levels in the liver and spleen. The absolute efficiency of LDFO is greater than 50% on a mole LDFO injected /mole iron removed from the liver (P<0.0004, n=4) and spleen (P<0.01, n=4). This is corroborated by an elevated iron accumulation in urine and feces from LDFO.

Conclusions

LDFO effectively removes iron from the liver and spleen with an overall molar efficiency > 50%. This high efficacy could lead to a dramatically improved treatment that increases compliance and provides substantially better management of iron overload than current treatments in patients suffering from iron overload conditions.

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