Sustained Release Multivesicular Liposomal (DepoFoam®) Formulation of IFN-α_{2b} that provides Sustained Plasma IFN-α_{2b} levels for Seven Days

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Objectives

The objective of our work was to develop a sustained-release DepoFoam® formulation of IFN-α2b that provides:

- **High IFN-α2b encapsulation efficiency (>85%)**
- **No loss of IFN-α2b bioactivity during manufacturing**
- **Maintenance of efficacious serum IFN-α2b levels in rats for 7 days**
- **Minimal post-administration release and low serum Cmax/Cmin ratio**
- **Long term storage stability at 2-8°C**

Methodology

Formulation Manufacture

IFN-α2b was encapsulated within DepoFoam particles using a high-efficiency double emulsification process that is applicable for use with both small molecules and biomolecules. To manufacture DepoIFN-α2b particles, an aqueous IFN-α2b solution was emulsified in an immiscible lipid solution to produce a W/O emulsion. The W/O emulsion was then emulsified with a second aqueous solution to produce a W/O/W particle suspension. The organic solvent was evaporated from the particles to produce multi-chambered DepoIFN-α2b particles (Figs 1a,b). The particle suspensions were then washed and stored in normal saline. After manufacture, DepoFoam formulations were characterized with respect to:

- **IFN-α2b encapsulation efficiency by RP-HPLC**
- **Structural integrity of the encapsulated IFN-α2b by SDS-PAGE (Invitrogen Corp)**
- **Bioactivity of the encapsulated IFN-α2b by cytopathic effect assay (CPE, PBL Biomedical Laboratories)**
- **Particle size distribution by laser light diffraction analysis (Horiba LA-910)**

DepoIFN-α2b Pharmacokinetics and Biocompatibility

DepoIFN-α2b formulations were evaluated in a rat model as follows: DepoIFN-α2b was administered by subcutaneous (SC) injection in the rat hindlimb. Blood was collected via the saphenous vein, and serum, isolated by centrifugation from the blood samples, was analyzed by ELISA (Bender MedSystems). The injection site was evaluated visually to determine biocompatibility of the DepoIFN-α2b formulations.

Long Term Storage Stability (2-8°C)

DepoIFN-α2b formulations stored at 2-8°C were evaluated at intervals up to 6 months to determine their physical and chemical stability. Physical stability was determined using microscopy, particle size distribution and analysis of IFN-α2b released from the particles (RP-HPLC). Chemical stability of the encapsulated IFN-α2b was determined using RP-HPLC and SDS-PAGE analysis.

Results

Formulation Characteristics

IFN-α2b was encapsulated in DepoFoam particles with 86% efficiency. Less than 1% of the IFN-α2b was free in the normal saline storage solution. DepoIFN-α2b particles were spherical (Fig 1b) and had monomodal particle size distribution (20um median diameter, Fig 2). The encapsulated IFN-α2b was shown by RP-HPLC and SDS-PAGE to have retained its chemical integrity (Figs 3, 4). The bioactivity (IU/mg) for encapsulated IFN-α2b was unchanged as measured by the cytopathic inhibition assay.

DepoIFN-α2b Pharmacokinetics and Biocompatibility

Rat plasma pharmacokinetics were determined for five replicate batches of the DepoIFN-α2b formulation (Fig 5). Following SC administration, only a small fraction of the encapsulated IFN-α2b was released from the particles. Thereafter, DepoIFN-α2b formulations released IFN-α2b in a near zero-order fashion for up to 7d. The Cmax occurred between 4-5 days. Accumulation of AUC was near linear for up to 7d (Fig 6). CPE analysis of IFN-α2b - containing rat serum samples showed that the IFN-α2b released from DepoIFN-α2b particles retained ~100% bioactivity.

DepoIFN-α2b formulations were well tolerated. There were no indications of irritation or erythema at the site of injection during the 7 day study period, nor at necropsy. Furthermore, the subcutaneous DepoIFN-α2b depots were not visually detectable at the site after 10 days.

Long Term Storage Stability

DepoIFN-α2b formulations were chemically and physically stable during 2-8°C storage for the duration of the monitoring period (6M). There was no detectable change in total IFN-α2b content nor particle size distribution of the DepoIFN-α2b formulations (Figs 7, 2). There was no detectable leakage of IFN-α2b from the formulations during storage. Unencapsulated (free) IFN-α2b never exceeded 1% during 6 months of storage.

SDS-PAGE analysis of samples stored for 6M showed that the encapsulated IFN-α2b was unchanged relative to an IFN-α2b solution control. IFN-α2b oligomerization was not detectable by Coomassie staining (Fig 4, LOD =1.8%). Silver staining of overloaded samples was required to visualize oligomers, and the extent of oligomerization in DepoIFN-α2b stored for 6M was indistinguishable from unencapsulated IFN-α2b solution (Fig 8).

Conclusions

- **IFN-α2b was encapsulated with 86% efficiency into DepoFoam particles. The particle suspensions were monomodal, and the median particle size was 20μm. The suspensions contained less than 1% free IFN-α2b. DepoIFN-α2b delivered bioactive IFN-α2b in a near-zero-order fashion for up to 7d in a rat subcutaneous model. DepoIFN-α2b was biocompatible in the rat.**
- **DepoFoam-encapsulated IFN-α2b retained its chemical integrity and bioactivity for at least 6 months during 2-8°C storage.**
- **DepoIFN-α2b was physically stable during 2-8°C storage. There was no change in particle size distribution nor percent free IFN-α2b during 6 months storage.**
- **DepoFoam encapsulation represents an attractive solution for the delivery of small molecules and biologics.**

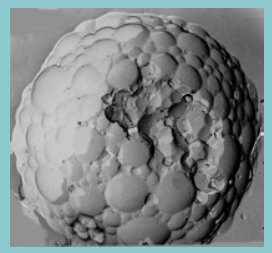

Fig 1a. Electron micrograph of DepoFoam particle

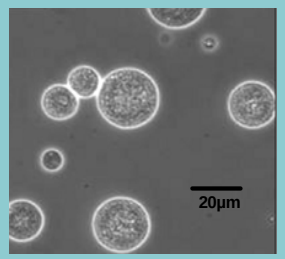

Fig 1b. Phase contrast micrograph of DepoFoam particles

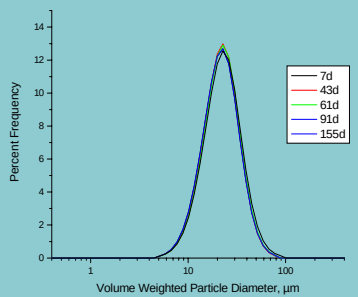

Fig 2. Particle size distribution of DepoIFN-α_{2b} suspension

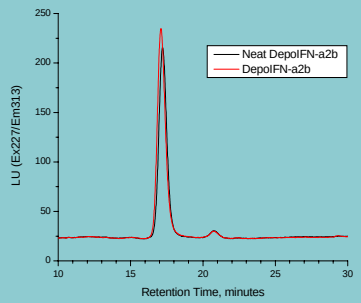

Fig 3. RP-HPLC chromatogram of encapsulated vs neat IFN-α_{2b}

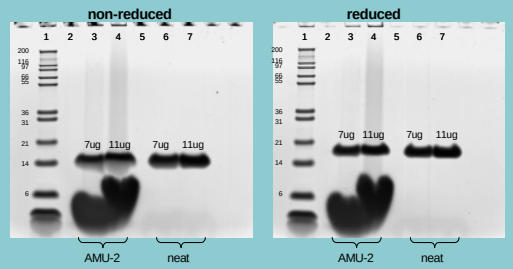

Fig 4. SDS-PAGE analysis of DepoIFN-a2b following 6M storage at 2-8°C (Coomassie stain)

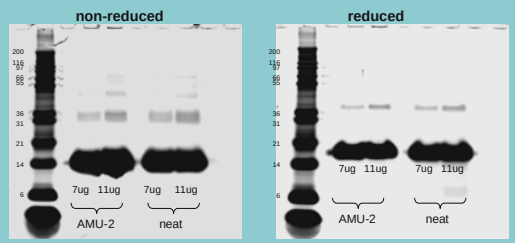

Fig 8. SDS-PAGE analysis of DepoIFN-a2b following 6M storage at 2-8°C (Silver stain)

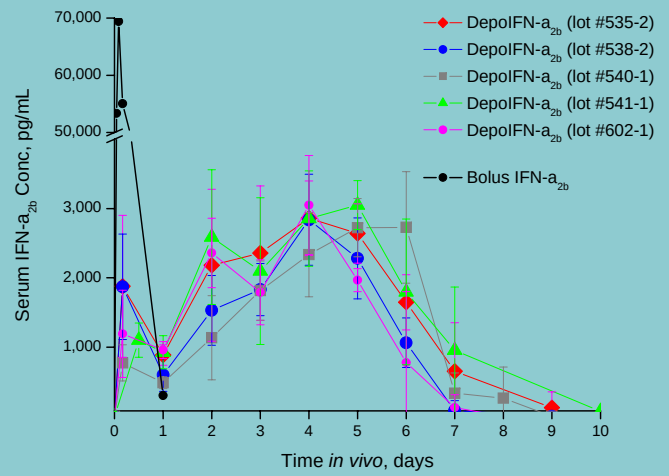

Fig 5. Rat serum IFN-a2b levels following administration of DepoIFN-a2b vs bolus IFN-a2b

DepoFoam formulations offer:

- **Efficient encapsulation of small molecules and biologics**
- **Precedence in two marketed products**
- **A well tolerated SC depot**
- **Near zero-order pharmacokinetics**
- **Long term stability**

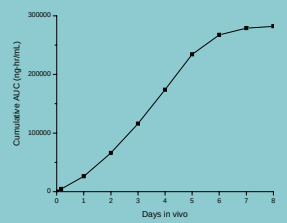

Fig 6. Cumulative AUC for IFN-a2b in rat serum following DepoIFN-a2b administration

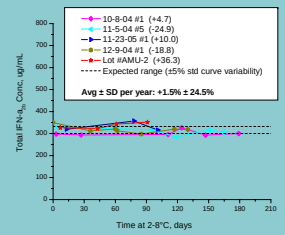

Fig 7. RP-HPLC analysis of DepoIFN-a2b during 6M storage at 2-8°C

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Sustained Release Multivesicular Liposomal (DepoFoam®) Formulation of IFN-α_{2b} that provides Sustained Plasma IFN-α_{2b} levels for Seven Days

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Conclusions

- IFN-α_{2b} was encapsulated with 86% efficiency into DepoFoam particles. The particle suspensions were monomodal, with median particle size of 20µm. The suspensions contained less than 1% free IFN-α_{2b}
- DepoIFN-α_{2b} delivered bioactive IFN-α_{2b} in a near-zero-order fashion for up to 7d in a rat subcutaneous model. DepoIFN-α_{2b} was biocompatible in the rat.
- DepoFoam-encapsulated IFN-α_{2b} retained its chemical integrity and bioactivity for at least 6M at 2-8°C.
- DepoIFN-α_{2b} was physically stable during 2-8°C storage. There was no change in particle size distribution nor percent free IFN-α_{2b} during 6 months storage.

DepoFoam encapsulation represents an attractive solution for the delivery of small molecules and biologics. DepoFoam formulations offer:

- Efficient encapsulation of small molecules and biologics
- Precedence in two marketed products (DepoCyt™ and DepoDur ™)
- A well tolerated SC depot
- Near zero-order pharmacokinetics
- Long term stability