LIQ865A, a Slow Release Microparticle Formulation of Bupivacaine, is Well-Tolerated and Does Not Interfere with Wound Healing after Subcutaneous Dosing in Rats and Minipigs

#1586

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INTRODUCTION and OBJECTIVE

LIQ865A is a bupivacaine (Bup) formulation developed by Liquidia Technologies, Inc. for the management of local post-operative pain using a proprietary process technology called PRINT® (Particle Replication In Non-wetting Templates). LIQ865A is 25 µm hexagonal particles comprised of approximately 55% bupivacaine and 45% poly(lactic-co-glycolic) acid (PLGA). Particles are suspended in a custom vehicle for subcutaneous (SC) administration. LIQ865A is designed to slowly release bupivacaine at the surgical site over 3 to 5 days providing a longer pain management solution as compared to the current state of the art while reducing the potential for systemic toxicity secondary to an increase in bupivacaine plasma concentrations.

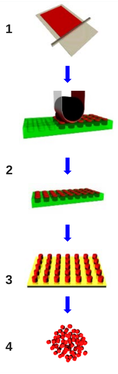
To support clinical testing, pivotal GLP studies were conducted in both Sprague Dawley rats and Yucatan miniature swine to assess local tolerability and the potential impact of LIQ865A on wound healing.

PRINT® Fabrication Process

During PRINT manufacturing, formulation components are formed into the desired shape and size using a molding process that produces a bulk powder consisting of particles of uniform size, shape, and composition. Several variables can be leveraged to produce a wide range of shapes, sizes, and composition.

The core process involves four basic steps:

1) Create a film of the desired composition on a delivery sheet.
2) Laminate the film with a mold template where the material fills the mold cavities.
3) Remove particles from the mold template.
4) Collect particles to create a particle suspension or dry powder.



STUDY DESIGN and METHODS

Single SC Administration Toxicity Study In Rats

Treatment	Bup Dose[a] (mg/kg)	Total Particle Dose (mg/kg)	Bup Conc. (mg/mL)	No. of Animals/Sex/Cohort			
				Necropsy Day 7	Necropsy Day 14	Necropsy Day 30	Necropsy Day 60
Vehicle	0	0	0	5	5	5	5
PLGA Placebo	0	62[b]	0	5	5	5	5
LIQ865A	20	36	16.7	5	5	5	5
LIQ865A	80	142	67	5	5	5	5

[a]Dosing volume = 1.2 mL/kg
[b]PLGA Placebo was mass-matched to the PLGA content in the 80 mg/kg LIQ865A dose group.

- **Regulatory Compliance:** GLP
- **Animals:** Sprague Dawley, ~9 weeks of age at Study Day 1
- **In-life data:** mortality, clinical signs, body weights, food consumption, clinical pathology
- **Dosing Site Observations:**
  - Day 1-3, daily after that for animals that showed continued injection site signs
  - Exam included observations for irritation, redness, edema/accumulation of fluid, scabbing
- **Post-mortem data:** gross pathology, organ weights, histopathology (dosing site tissues including skin, subcutis, underlying muscle and inguinal lymph nodes)
- **Toxicokinetics:**
  - No. of animals (satellite groups): 3/sex/time point
  - Blood Collection: direct venipuncture of jugular vein (5-6 samples per rat)
  - Blood Collection Time Points:
    - Vehicle, Placebo: 0.5, 4 hrs following administration
    - LIQ865A: 0.5, 1.25, 2.5, 4, 6, 8, 24, 30, 48, 72, 96 hrs following administration
  - Bioanalytical Method: Validated LC-MS/MS assay (AIT Bioscience)
  - PK Analysis: Phoenix WinNonlin, Ver. 6.3 (Pharsight Corporation)

Single SC Administration Toxicity Study in Minipig Full-Thickness Incisional Model

Treatment	Bup Dose[a] (mg/kg)	Total Particle Dose (mg/kg)	Bup Conc. (mg/mL)	No. of Animals/Sex/Cohort		
				Necropsy Day 3	Necropsy Day 14	Necropsy Day 28
Sham	NA	NA	NA	3	3	3
Vehicle	0	0	0	3	3	3
LIQ865A	6	10.8	15	3	3	3
LIQ865A	18	32.4	45	3	3	3
LIQ865A	36	64.9	90	3	3	3

[a]Dosing volume = 0.4 mL/kg

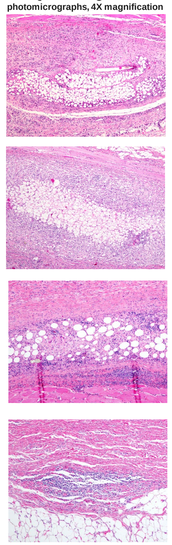
- **Regulatory Compliance:** GLP
- **Animals:** Yucatan miniature swine (*Sus scrofa*), 3-5 months at Study Day 1
- **Surgery and Dosing:**
  - 10-cm full-thickness incisional wound on left dorsum perpendicular to midline.
  - Half of total volume administered on each side of the incision directly thru the open incision
  - Following dosing, incision was sutured closed and bandaged
- **In-life data:** mortality, clinical signs, body weights, clinical pathology
- **Dosing/Incision Site Observations:**
  - Draize Scoring daily for 7-10 days and prior to termination
- **Post-mortem data:** gross pathology, organ weights, histopathology (standard tissues plus incision sites and inguinal lymph nodes)
- **Toxicokinetics:**
  - Blood Collection: direct venipuncture of jugular vein (3-4 blood samples per pig)
  - Blood Collection Time Points:
    - Necropsy Day 3 Cohort: predose, 0.25, 48 hrs following administration
    - Necropsy Day 14 Cohort: 1, 8, 72 hrs following administration
    - Necropsy Day 28 Cohort: 2, 4, 24, 96 hrs following administration
  - Bioanalytical Method: Validated LC-MS/MS assay (AIT Bioscience)
  - PK Analysis: Phoenix WinNonlin, Ver. 7.0 (Pharsight Corporation)

In Rats, LIQ865A was Well-Tolerated with No Adverse Findings

In Minipigs, LIQ865A was Well-Tolerated with No Effect on Wound Healing

In both Rats and Minipigs, tissue response to LIQ865A was a continuum progressing from the initial injury and an acute cellular response through a granulomatous inflammatory response to resolution.

LIQ865A High Dose
photomicrographs, 4X magnification



Rat SC Injection Site Histopathology

Day 7
- Similar changes for Placebo and LIQ865A.
- Inflammatory response and fibrosis localized to subcutis, injection site.
- Both Placebo and LIQ865A particles present.

Day 14
- Vascularized fibrous connective tissue, lymphocytes, macrophages, giant cells and fatty infiltrates at similar or slightly decreased levels compared to Day 7.
- Particle deposits were decreased in incidence and/or deposit extent compared to Day 7.

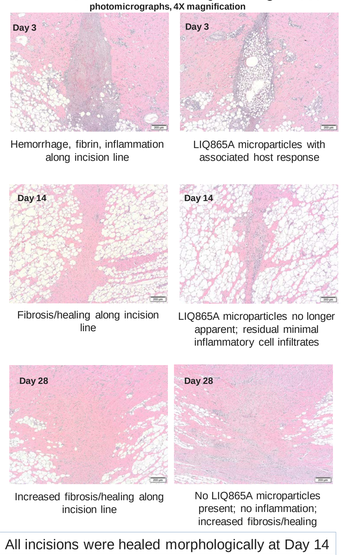
Day 30
- Particle deposits no longer present.
- Persisting infiltrates (lymphocytes, macrophages, giant cells, fibrosis, fat) were decreased in incidence and severity compared to Day 14.
- No giant cells observed in low dose group.

Day 60
- Inflammation persisted in 1-3 rats / treatment group consisting of small aggregates of lymphocytes with rare neutrophils or plasma cells.
- Macrophages and giant cells were not present.

Minipig Incision Site Histopathology – Incidence and Severity

DAY 3	Males					Females				
	Sham	Vehicle	LIQ865A			Sham	Vehicle	LIQ865A		
LIQ865A (mg/kg) =	0	0	6	18	36	0	0	6	18	36
Cellular infiltrates assoc. with particles, subcutis										
Incidence	0/3	0/3	3/3	3/3	3/3	0/3	0/3	3/3	2/3	3/3
minimal	0	0	1	1	2	0	0	0	1	3
mild	0	0	2	2	1	0	0	3	1	0
Mononuclear cell infiltration, adipose/subcutis										
Incidence	0/3	1/3	0/3	0/3	3/3	0/3	0/3	0/3	0/3	0/3
minimal	0	1	0	0	0	0	0	0	0	0
Acute inflammation, subcutis/incision line										
Incidence	3/3	3/3	3/3	3/3	3/3	0/3	3/3	3/3	3/3	3/3
minimal	1	2	3	1	1	2	2	2	2	2
mild	2	1	0	2	2	1	1	1	1	1
Microparticles, subcutis adjacent to incision line										
Present	0/3	0/3	3/3	3/3	3/3	0/3	0/3	3/3	3/3	3/3
DAY 14										
Cellular infiltrates assoc. with particles, subcutis										
Incidence	0/3	0/3	0/3	1/3	0/3	0/3	0/3	0/3	0/3	2/3
mild	0	0	0	1	0	0	0	0	0	0
moderate	0	0	0	0	0	0	0	0	0	2
Granulomatous Infiltration, subcutis										
Incidence	0/3	0/3	3/3	3/3	3/3	0/3	0/3	3/3	3/3	3/3
minimal	0	0	2	1	1	0	0	2	0	1
mild	0	0	1	2	2	0	0	1	3	1
moderate		0	0	0	0	0	0	0	0	1
Foreign-body microgranuloma(s)										
Incidence	1/3	2/3	1/3	1/3	2/3	1/3	2/3	3/3	1/3	0/3
minimal	1	2	1	1	2	1	2	3	1	0
Microparticles, subcutis adjacent to incision line										
Present	0/3	0/3	0/3	1/3	0/3	0/3	0/3	0/3	0/3	2/3
Day 28										
Collagen degeneration, subcutis										
Incidence	0/3	1/3	0/3	1/3	0/3	0/3	0/3	0/3	0/3	0/3
minimal	0	0	0	1	0	0	0	0	0	0
mild	0	1	0	0	0	0	0	0	0	0
Granulomatous Infiltration, subcutis										
Incidence	0/3	0/3	3/3	2/3	2/3	0/3	0/3	0/3	3/3	3/3
minimal	0	0	3	2	1	0	0	0	3	2
mild	0	0	0	0	1	0	0	0	0	1
Foreign-body microgranuloma(s)										
Incidence	0/3	0/3	1/3	0/3	1/3	3/3	1/3	1/3	1/3	2/3
minimal	0	0	1	0	1	3	1	1	1	2

Vehicle **LIQ865A High Dose**
photomicrographs, 4X magnification



Hemorrhage, fibrin, inflammation along incision line

LIQ865A microparticles with associated host response

Fibrosis/healing along incision line

LIQ865A microparticles no longer apparent; residual minimal inflammatory cell infiltrates

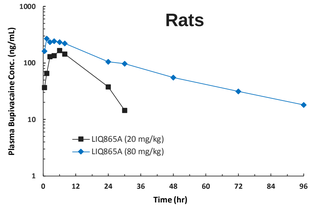
Increased fibrosis/healing along incision line

No LIQ865A microparticles present; no inflammation; increased fibrosis/healing

All incisions were healed morphologically at Day 14

In Rats and Minipigs, LIQ865A Administration over a 4 to 6-fold Bup Dose Range Resulted in ≤2X Increase in Bup C_{max}

Rats

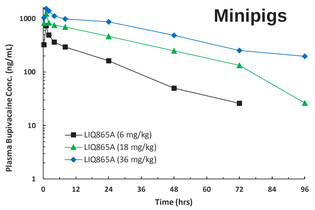


- Bup C_{max} and AUC increased in a less than dose proportional manner compared to increasing Bup dose level
- No Bup burst release (1.25 – 6 hr T_{max}).

Treatment	Dose (mg/kg)	C_{max} (ng/mL)	T_{max} (hr)	$t_{1/2}$ (hr)	AUC_{inf} (hr*ng/mL)
LIQ865A	20	168	6.0	6.6[a]	3040[a]
LIQ865A	80	283	1.25 - 6	27.5	8760

[a]Females only; Terminal rate constant could not be adequately estimated for males.

Minipigs



- Increase in Bup C_{max} was less than dose proportional while AUC was generally dose proportional
- No Bup burst release (1 hr T_{max})

Treatment	Dose (mg/kg)	C_{max} (ng/mL)	T_{max} (hr)	$t_{1/2}$ (hr)	AUC_{inf} (hr*ng/mL)
LIQ865A	6	739	1.0	26.5	11600
LIQ865A	18	1230	1.0	14.0	31200
LIQ865A	36	1530	1.0	39.8	65800

CONCLUSION

In both rats and minipigs, the local changes associated with LIQ865A were consistent with a degradable foreign body response and what is reported for Bupivacaine. No novel findings or safety concerns were identified. The high dose level was considered the NOAEL in both studies.