LIQ865A Produces a Slow Controlled Release of Bupivacaine after Subcutaneous Dosing in Rats and Minipigs

#1600

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INTRODUCTION and OBJECTIVE

LIQ865A is a bupivacaine particle formulation developed by Liquidia Technologies, Inc. for the management of local post-operative pain. LIQ865A is manufactured using a proprietary process technology called PRINT® (Particle Replication In Non-wetting Templates) producing 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 surgical site infiltration or 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 without increasing the potential for systemic toxicity secondary to an increase in plasma concentrations of bupivacaine.

To support clinical testing, single dose bupivacaine (Bup) pharmacokinetics (PK) from subcutaneously administered LIQ865A, Marcaine™ (Bup HCl solution) and Exparel® (liposomal bupivacaine) were studied in Sprague Dawley rats and Yucatan miniature swine.

STUDY DESIGN and METHODS

- LIQ865A formulations were prepared just prior to administration.
- **Single SC Administration PK in Sprague Dawley Rats (Rat SC PK) :**
  - All rats received a single SC administration of test article (LIQ865A, Exparel, Marcaine)
  - Blood samples were collected via jugular venipuncture
  - Sparse sampling method (samples collected at 5 or 6 time points per rat)
- **Miniature Swine (Yucatan):**
  - Blood samples were collected via indwelling jugular catheter
  - **Single SC Administration PK (Pig SC PK) and SC LIQ865A and Lidocaine Single SC Administration (Pig 865A/Lido PK):**
    - 3-6 SC injections along virtual 5-cm incision line
    - Lidocaine was administered 5 min prior to LIQ865A along the virtual incision line
  - **Incisional Wound Model PK (Pig Wound PK):**
    - 10-cm full-thickness incisional wound on left dorsum perpendicular to midline
    - Half of total volume administered subcutaneously on each side of the incision directly thru the open incision
    - Following dosing, incision was sutured closed and bandaged

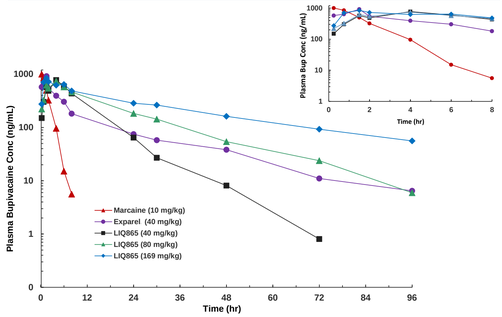
Study Parameters	Rat SC PK	Pig SC PK	Pig Wound PK	Pig 865A/Lido PK
Treatment	Marcaine Exparel LIQ865A	Marcaine Exparel LIQ865A	Marcaine Exparel LIQ865A	LIQ865A LIQ865A/Lidocaine
Bup Doses (mg/kg)	10 40 40, 80, 169	2 4 2, 4, 6	2 6 6, 18, 36	18 18/4
Bup Conc. (mg/mL)	7.5 13.3 26.7, 53.3, 113	5 13.3 6.7, 13.3, 20.0	5 13.3 15, 45, 90	45 45/20
Dosing Volume (mL/kg)	1.35 3.0 1.5	0.4 0.3 0.3	0.4 0.45 0.4	0.4 0.4/0.2
No. of Animals (n/sex/group/time pt)	3 (Marcaine, males only)	3 (males only)	3	4
Blood Sampling Times (Hours Post Dose)	0.25, 0.75, 1.5, 2, 4, 6, 8, 24, 30, 48, 72, 96 hrs	0.083, 0.5, 1, 2, 4, 6, 8, 12, 24, 48, 72, 96, 120 hrs	0, 0.083, 0.25, 0.5, 1, 2, 4, 8, 24, 48, 72, 96, 120, 144 hrs	0, 0.25, 0.5, 1, 2, 4, 8, 24, 48, 72, 96, 120 hrs

PK Analysis: Phoenix WinNonlin, ver. 6.3 or 6.4

Bioanalytical Methodology (AIT Biosciences):
- Validated Method: Solid phase extraction and LC-MS/MS instrumental analysis
- Range: 2 – 2000 ng/mL

RESULTS

In Rats, Plasma Bupivacaine C_{max} did not Increase with Increasing LIQ865A Dose



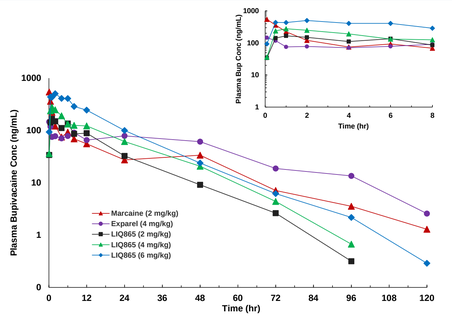
Mean Plasma Bup PK Parameters

Treatment Group	Dose (mg/kg)	C_{max} (ng/mL)	T_{max} (hr)	$t_{1/2}$ (hr)	AUC_{inf} (hr*ng/mL)
Marcaine	10	1004	0.3	1.0	1864
Exparel	40	919	1.5	15.3	7570
LIQ865A	40	777	4.0	7.6	9105
LIQ865A	80	743	4.0	16.1	13591
LIQ865A	169	844	1.5	28.1	23737

Summary: SC Administration In Rats

- Bup C_{max}, when administered as LIQ865A, was lower than the C_{max} for Exparel or Marcaine administered at a similar or lower dose.
- Bup exposure, as measured by AUC_{inf}, increased in a less than dose proportional manner.
- Bup half-life was generally dose proportional when administered as LIQ865A.
- There were no gender differences in Bup exposure.

In Minipigs, LIQ865A Dosed 3 Times Higher than Marcaine Resulted in Lower Bupivacaine C_{max} Values



Mean Plasma Bup PK Parameters

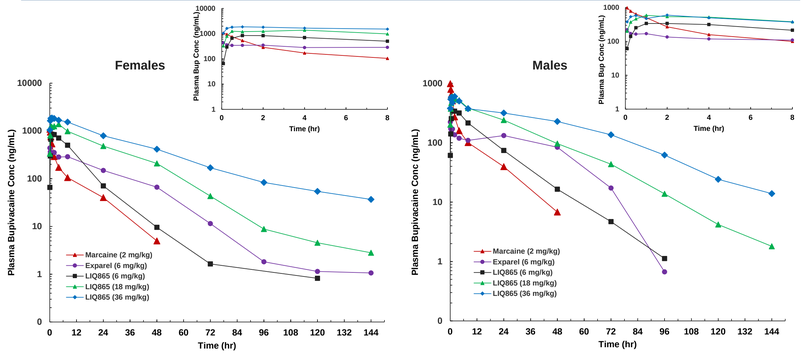
Treatment Group	Dose (mg/kg)	C_{max} (ng/mL)	T_{max}* (hr)	$t_{1/2}$ (hr)	AUC_{inf} (hr*ng/mL)
Marcaine	2	545	5 min	19.7	3010
LIQ865A	2	174	1.0	14.8	2780
Exparel	4	146	5 min	24.4	5050
LIQ865A	4	297	1.0	10.7	4400
LIQ865A	6	511	2.0	13.7	8320

* median values

Summary: SC Administration In Male Minipigs

- At 4 mg/kg, Exparel had the fastest absorption with a T_{max} at 5 min compared to LIQ865A at 1 hr.
- SC administration of low doses of LIQ865A resulted in dose proportional increases in Bup Exposure (C_{max}, AUC).
- Bup exposure (C_{max}, AUC) was generally dose proportional when administered as LIQ865A.
- Bup half-life did not change with increasing LIQ865A dose.

In a MInipig Full-Thickness Incisional Model, SC Administration of LIQ865A Over a 6-Fold Bupivacaine Dose Range Resulted in Only a 2X Increase in Bupivacaine C_{max}



Mean Plasma Bup PK Parameters

Treatment Group	Dose (mg/kg)	Sex	C_{max} (ng/mL)	T_{max}* (hr)	$t_{1/2}$ (hr)	AUC_{last} (hr*ng/mL)	AUC_{inf} (hr*ng/mL)
Marcaine	2	M	984	5 min	9.5	3670	3780
		F	1080	5 min	8.0	3830	3930
Exparel	6	M	224	5 min	8.3	6890	7890
		F	443	5 min	19.8	9380	9980
LIQ865A	6	M	359	2.0	9.0	5940	6020
		F	865	2.0	8.3	11000	9480
LIQ865A	18	M	604	1.0	18.2	15500	15700
		F	1450	2.0	13.8	33400	33500
LIQ865A	36	M	620	2.0	20.0	24200	24600
		F	1920	1.0	32.9	59300	61100

* Median values. M: male; F: female.

LIQ865A Dose Proportionality

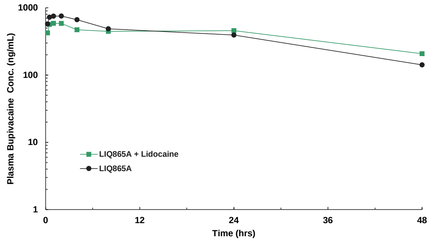
Treatment Group	Dose (mg/kg)	Increase[a]	Male			Female		
			C_{max}	AUC_{last}	AUC_{inf}	C_{max}	AUC_{last}	AUC_{inf}
LIQ865A	6	-	--	--	--	--	--	--
LIQ865A	18	3.0	1.7	2.6	2.6	1.7	3.0	3.5
LIQ865A	36	2.0	1.0	1.6	1.6	1.3	1.8	1.8
	overall[b]	6.0	1.7	4.1	4.1	2.2	5.4	6.4

[a]Fold increase between adjacent doses; [b]Fold increase over entire dose range

Summary: SC Administration In MInipig Incisional Model

- At 6 mg/kg, Exparel and LIQ865A produced similar Bup AUCs and similar half-life.
- T_{max} was earliest for Exparel and Marcaine (5 min) compared to 1 to 2 hours for LIQ865A.
- Bup C_{max} was less than dose proportional while AUC was generally dose proportional.
- Mean Bup C_{max} was similar at 18 and 36 mg/kg within LIQ865A genders.
- Gender differences in Bup exposure were evident for LIQ865A and Exparel.

SC Administration of Lidocaine to Minipigs 5 min prior to LIQ865A Did Not Result in a Burst Release of Bupivacaine



Mean Plasma Bup PK Parameters

Treatment	C_{max} (ng/mL)	T_{max}* (hr)	AUC_{last} (hr*ng/mL)	AUC_{inf} (hr*ng/mL)
LIQ865A	782	1.50	22200	22400
LIQ865A + Lidocaine	613	1.50	25400	26700

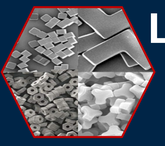
*median value

Summary: SC Co-Administration of LIQ865A with Lidocaine in MInipigs

- No burst release of Bup when LIQ865A was dosed 5 min after Lidocaine as evidenced by a slightly lower mean C_{max} value than LIQ865A alone.
- Lidocaine did not change the Bup T_{max}.
- No gender differences were seen in Bup exposure.

CONCLUSION

- **SC Administration of LIQ865A results in a slow controlled release of Bup without an initial burst following SC administration in rats and miniature swine**:
  - ✓ Delayed T_{max} compared to Marcaine or Exparel
  - ✓ Less than dose proportional increase or no increase in C_{max} with increasing dose
  - ✓ Increased AUC with increasing LIQ865A dose
  - ✓ Prolonged $t_{1/2}$ with increasing LIQ865A dose



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.

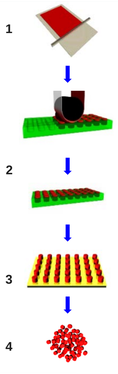
To support clinical testing, pivotal GLP studies were conducted in both Sprague Dawley rats and Yucatan miniature swine to assess local tolerability and the potential impact of LIQ865A on wound healing.

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

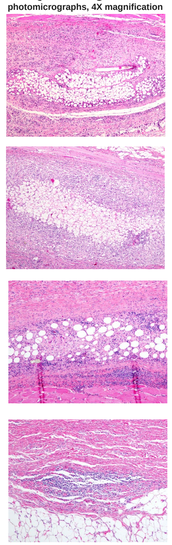
- **Regulatory Compliance:** GLP
- **Animals:** Yucatan miniature swine (*Sus scrofa*), 3-5 months at Study Day 1
- **Surgery and Dosing:**
  - 10-cm full-thickness incisional wound on left dorsum perpendicular to midline.
  - Half of total volume administered on each side of the incision directly thru 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.

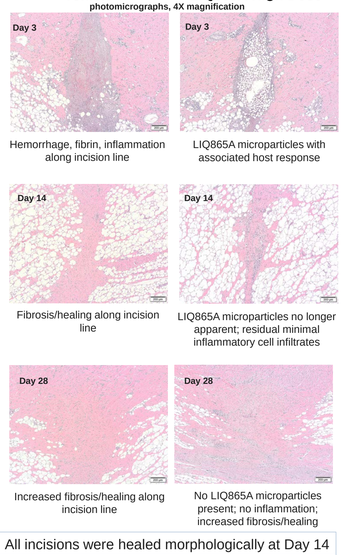
Day 30
- Particle deposits no longer present.
- Persisting infiltrates (lymphocytes, macrophages, giant cells, fibrosis, fat) were decreased in incidence and severity compared to Day 14.
- No giant cells observed in low dose group.

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

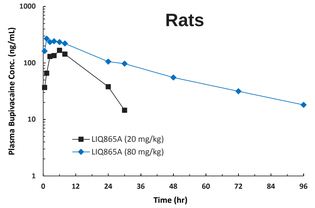
Increased fibrosis/healing along incision line

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

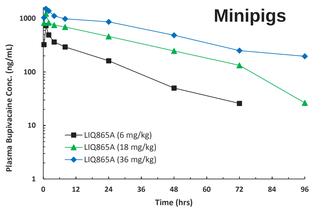


- Bup C_{max} and AUC increased in a less than dose proportional manner compared to increasing Bup dose level.
- No Bup burst release (1.25 – 6 hr T_{max}).

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.