The Safety Evaluation of Multilamellar Vesicles Formulated Ropivacaine, TLC590, Administered by Local Injection in Incisional Wounds in Rats and Miniature Swine

6921

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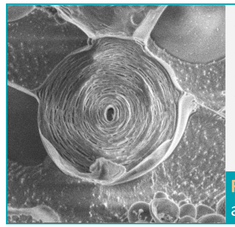
Introduction

Improvement in postoperative pain control plays an important role in the quality of patient recovery and satisfaction. Multilamellar vesicles formulated ropivacaine, TLC590 (Figure 1), is a clinical stage, sustained-release ropivacaine formulation being investigated for postsurgical analgesia via local injection. Supporting toxicology studies after subcutaneous or intramuscular administration in acute incisional wounds have been conducted in rats and miniature swine.



TLC590 is a two-vial system comprising of one vial of sterile TLC590 lyophilized cake and one vial of sterile TLC590 reconstitution solution. The major components include ropivacaine, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine and cholesterol.

Figure 1 Electron microscopic image of TLC590, a multilamellar vesicle structure.

Materials and Methods

Rat study

Groups of 12 to 14 male rats received either TLC590 at low dose, middle dose or high dose, vehicle or normal saline along the incision site (Table 1). Body weights and clinical observations were recorded daily. Six rats in each group were sacrificed on Day 3 and the remaining rats were sacrificed on Day 14 for gross observations and histopathological examination of skin (injection site).

Miniature swine study

Groups of 8 miniature swine/sex received either TLC590 at low dose, middle dose or high dose, vehicle or normal saline and groups of 4 miniature swine/sex received Naropin® along the inguinal surgical hernia site with polypropylene mesh implantation (2.5 × 5 cm) (Table 1). The animals were monitored for mortality observations, body weights, feed consumption and wound observations using modified Draize scores and wound condition scores. Four miniature swine/sex/group were sacrificed on Day 4 and the remainder were sacrificed on Day 29 for macroscopic examinations. Endpoints included necropsy and tissue collection, clinical pathology, organ weights and histopathological examination of a full tissue list including dosing site, ilioinguinal nerve and inguinal lymph nodes. Toxicokinetic samples were taken at designated time points and analyzed using LC-MS/MS.

Table 1 Description of treatment group design and dose levels.

Rat study Group	Dose of ropivacaine		Number of Animals	
	mg/kg	mL/kg	Necropsy Day 3	Necropsy Day 14
A) TLC590 (Low dose)	20	1	6/Male	7/Male
B) TLC590 (Middle dose)	80	4	6/Male	8/Male
C) TLC590 (High dose)	200	10	6/Male	8/Male
D) Vehicle	0	10	6/Male	6/Male
E) Normal saline	0	10	6/Male	6/Male
Miniature swine study Group	**Dose of ropivacaine**		**Number of Animals**	
	mg/kg	mL/kg	Necropsy Day 4	Necropsy Day 29
A) TLC590 (Low dose)	8	0.42	4/sex	4/sex*
B) TLC590 (Middle dose)	30	1.58	4/sex	4/sex*
C) TLC590 (High dose)	40	1.58	4/sex	4/sex*
D) Vehicle	0	1.58	4/sex	4/sex
E) Normal saline	0	1.58	4/sex	4/sex
F) Naropin®	8	0.91	4/sex*	—

*, Toxicokinetic study groups

Results and Discussion

Rat study

All rats survived until scheduled termination. There were no significant differences in body weights, body weight changes, clinical observations and gross observations among all group animals. Results in rats suggested no TLC590-related and dose-related toxic effects. The histopathological examination of skins revealed no treatment-associated effect during the wound healing process (Table 2).

Table 2 Summary incidence and mean severity score of the microscopic finding in rats.

Time point		Day 3					Day 14				
Group		A	B	C	D	E	A	B	C	D	E
n		6	6	6	6	6	7	8	6	5[a]	6
Edema	TI	6/6	6/6	6/6	6/6	6/6	5/7	5/8	2/6	1/5	6/6
	MSS	1.83	1.67	1.67	2.00	1.67	0.857	0.625	0.333	0.200	1.00
Vascularity	TI	6/6	3/6	3/6	4/6	4/6	5/7	8/8	4/6	4/5	6/6
	MSS	1.67	1.00	0.833	1.17	1.33	0.714	1.50	0.833	1.00	1.00
Inflammatory reaction	TI	6/6	6/6	6/6	6/6	6/6	7/7	8/8	5/6	5/5	6/6
	MSS	2.00	2.33	1.83	2.33	1.33	1.14	1.38	0.833	1.00	1.00
Collagenization	TI	6/6	6/6	6/6	6/6	6/6	7/7	8/8	6/6	5/5	6/6
	MSS	1.50	2.50	1.67	1.67	1.83	2.86	2.63	3.00	3.00	2.83

[a], one animal was excluded since the incision site was injured by animal on Day 5.
TI, total incidence, which is represent by number of animal(s) with finding per number of animals examined in each group; MSS, mean severity score, which is calculated by dividing sum of severity scores (0, none; 1, mild; 2, moderate; 3, severe) by number of animals examined in each group.

Miniature swine study

No significant differences in body weights, body weight changes and clinical observations among all treatment groups in miniature swine were observed. The peak reactions, by Draize scoring, of erythema and edema on the dosing site in post-surgery monitoring appeared primarily on Day 2 or Day 3 in all groups for both genders and most of the reactions were resolved by Day 8. Similar results were observed in wound site assessment. Clinical pathology evaluations, organ weights, organ weight ratios and histopathological evaluations (Table 3) demonstrated that there were no patterns or trends in differences between treatment groups or genders indicating a correlation to TLC590 treatments either on Day 4 or Day 29.

Table 3 Summary scores (range) and incidence of the microscopic findings in male (top) and female (bottom) miniature swine at dosing site (mesh with tissues).

Time point		Day 4						Day 29				
Group		A	B	C	D	E	F	A	B	C	D	E
Fibroplasia	Range	0-2	0-2	1	1	0-2	0-2	1-2	1-2	2-3	0-3	1-3
	TI	3/4	3/4	4/4	4/4	1/4	3/4	4/4	4/4	4/4	3/4	4/4
Hemorrhage	Range	1-2	1	1-2	1-2	1-2	1-3	0	0	0	0-1	0
	TI	4/4	4/4	4/4	4/4	4/4	4/4	0/4	0/4	0/4	1/4	0/4
Inflammation	Range	1-2	1-2	1-2	1	1-2	1-2	1	1-2	1-2	0-1	1-2
	TI	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	2/4	4/4
Necrosis	Range	2-3	2-3	1-3	1-3	1-2	2-3	0	0	0	0	0
	TI	4/4	4/4	4/4	4/4	4/4	4/4	0/4	0/4	0/4	0/4	0/4
Time point		**Day 4**						**Day 29**				
Group		**A**	**B**	**C**	**D**	**E**	**F**	**A**	**B**	**C**	**D**	**E**
Fibroplasia	Range	1-2	1-2	1-2	1-2	1-2	2	1-2	1-3	1-2	1-2	0-3
	TI	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	3/4
Hemorrhage	Range	1-3	1-2	1-2	1-2	1-2	2-3	0	0	0	0	0
	TI	4/4	4/4	4/4	4/4	4/4	4/4	0/4	0/4	0/4	0/4	0/4
Inflammation	Range	1-2	1-2	1-2	1-2	1-2	2-3	1	1	1-2	1	1
	TI	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4
Necrosis	Range	2-3	2-3	2-4	2	1-2	3-4	0	0	0	0	0-2
	TI	4/4	4/4	4/4	4/4	4/4	4/4	0/4	0/4	0/4	0/4	1/4

Histopathological score: 0, none; 1, ≥ minimal; 2, ≥ mild; 3, ≥ moderate; 4, ≥ market; 5, ≥ severe.
TI, total incidence, which is represent by number of animal(s) with finding per number of animals examined in each group.

- These changes at dosing site were considered to be typical changes following a skin incision and were considered to represent normal reactions and findings associated with normal wound healing.
- All microscopic changes of dosing sites and organ findings were unrelated to TLC590 exposure.

Toxicokinetic results demonstrated that there were varying gender differences and the systemic exposure increased accordingly along with dose level increases, but less that dose-proportionally (Figure 2 and Table 4).

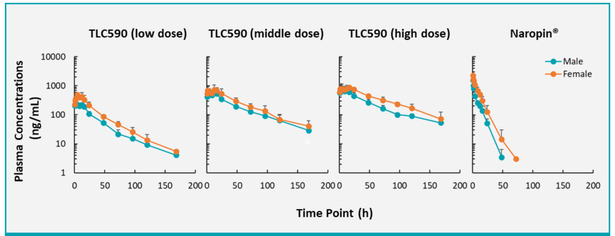


Figure 2 Mean plasma concentration versus time after a single local dose administration of TLC590 or Naropin® in hernia repairing model in miniature swine.

Table 4 The pharmacokinetic parameters after local injection of TLC590 and Naropin® in miniature swine.

Parameters	Unit	A	B	C	F
C_{max}	ng/mL	372 ± 148	737 ± 253	876 ± 280	1900 ± 448
t_{max}	h	4.78 ± 4.71	13.0 ± 6.61	8.03 ± 6.28	0.25 ± 0
$t_{1/2}$	h	36.2 ± 19.5	45.5 ± 19.0	52.8 ± 20.2	6.1 ± 1.2
$AUC_{0\text{-last}}$	ng × h/mL	11900 ± 4200	34400 ± 8350	48100 ± 13900	12300 ± 5620
$AUC_{0\text{-}\infty}$	ng × h/mL	12100 ± 4200	37000 ± 8700	53400 ± 12700	12300 ± 5620

Data represent mean value ± standard deviation. The data of TLC590 groups were obtained from the cohort Day 29 and the data of Naropin® group were obtained from the cohort Day 4.

- The C_{max} after injection of TLC590 (from 372 to 876 ng/mL when dosed from 8 mg/kg to 40 mg/kg) was significantly decreased compared with that after administration of Naropin® (1900 ng/mL when dosed 8 mg/kg); and the $t_{1/2}$ after injection of TLC590 (36.2 to 52.8 h) was significantly prolonged compared with that of Naropin® (6.1 h).

Conclusions

- A NOAEL of TLC590 is above 40 mg/kg for a single local administration in miniature swine, which the estimated human equivalent dose is 2270 mg/human (60 kg).
- These data demonstrate the safety and local tolerance of TLC590 in clinically relevant models and support the first-in-human trial of TLC590 by the wound infiltration route.
- A Phase I/II clinical trial to evaluate the safety, tolerability, pharmacokinetics and efficacy of TLC590 in patients was completed.

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