REL-1017 (esmethadone hydrochloride) demonstrates neither toxic effects on maternal health nor teratogenic effects in pregnant rats

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INTRODUCTION

- REL-1017 (esmethadone hydrochloride) is a safe and well tolerated[1] novel uncompetitive N-methyl-D-aspartate receptor (NMDAR) channel blocker with a preference for pathologically hyperactive GluN1-GluN2D NMDAR channels[2].
- REL-1017 has twenty-fold lower affinity at the mu-opioid receptor than levo-methadone[3] and lacks clinically meaningful opioid agonist actions[4-7].
- REL-1017 retains potential neuroplasticity and therapeutic effects without dissociative effects[1,4,5,6,7,8] and does not cause potentially neurotoxic Olney's brain lesions[9], unlike higher potency NMDAR blockers[9,10].
- REL-1017 is currently in Phase 3 clinical trials for the treatment of Major Depressive Disorder (MDD)[11].
- Preclinical data obtained from studies on well-established experimental models, indicated that REL-1017 did not show any appreciable evidence of abuse potential.

OBJECTIVE

REL-1017 (esmethadone hydrochloride) is a novel uncompetitive NMDAR channel blocker currently in Phase 3 clinical trials for the treatment for MDD. Because a proportion of patients affected by MDD are young and in child-bearing age, it is important to ascertain the developmental toxicity and teratogenic potential of REL-1017.

The objective of this study was to determine potential developmental toxicity, including teratogenic potential, of REL-1017 in pregnant Sprague Dawley (SD) rats.

METHODS

Sprague Dawley pregnant rats were treated daily via oral gavage from gestation day 6 through 17 with vehicle or REL-1017 at 10, 20, and 40 mg/kg/day (n= 80 total, n=20 rats/group). All the pregnant rats were evaluated for maternal survival, clinical findings (evaluation of the skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs and feet, as well as evaluation of respiration), ovarian and uterine parameters (mean number of corpora lutea, implantation sites, viable fetuses/litter size, resorptions, and pre- and post-implantation loss), and maternal macroscopic findings were assessed at any dose level evaluated. Each of the 468 fetuses (117 ± 6.5 for each group; mean ± SD) were individually weighed, sexed, tagged, and examined for external malformations and variations.

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DISCLOSURES

This research was sponsored by Relmada Therapeutics, Inc.
Drs. Pappagallo, Folli, and Manfredi are paid consultants for Relmada Therapeutics. Dr. Manfredi is an inventor on esmethadone patents and other patents and patent applications.

RESULTS

In this study, REL-1017 did not affect maternal survival, clinical findings, ovarian and uterine parameters, or maternal macroscopic findings at any dose level evaluated. Similarly, no effects of REL-1017 at any doses were observed on fetal sex ratios, fetal body weights, external, visceral, or skeletal development.

Table 1. Summary of Maternal Survival, Pregnancy Index, and Animals with Viable Fetuses

Sex: Female Day(s) Relative to Mating (Litter: A)	0 mg/kg/day (vehicle)	10 mg/kg/day (REL-1017)	20 mg/kg/day (REL-1017)	40 mg/kg/day (REL-1017)
Females in Study (n)	20	20	20	20
Not Pregnant (n)	1	0	1	0
Pregnant (n)	19	20	19	20
Pregnancy Index [f]*	95.0	100.0	95.0	100.0
Females w/ Viable Fetuses GD 20 (n)	19	20	19	20

*[f] = Fisher's Exact Test with Stepdown Sidak

Maternal survival, Pregnancy index, as well as animals with viable fetuses at the end of gestation were not affected by REL-1017 administered at 10, 20, and 40 mg/kg/day as compared to control vehicle. *(Table 1)*

Table 2. Summary of Maternal and Developmental Observations at Uterine Examination

Sex: Female Day(s) Relative to Mating (Litter: A)		0 mg/kg/day (vehicle) N = 19	10 mg/kg/day (REL-1017) N=20	20 mg/kg/day (REL-1017) N=19	40 mg/kg/day (REL-1017) N=20
# of Corpora Lutea*	Mean SD	15.2 1.68	14.1 1.83	13.6 2.54	13.5 1.88
# of Implantations/Animal**	Mean SD	13.5 1.5	12.9 1.09	12.1 2.53	12.2 2.82
% Pre-implantation Loss***	Mean SD	10.33 8.912	8.10 8.598	11.49 12.237	10.63 17.164
# of Viable Fetuses**	Mean SD	13.1 1.91	12.2 1.2	11.4 2.46	11.4 3.39
% of Live Male Fetuses in Litter***	Mean SD	44.5 12.91	54.3 13.41	54.0 14.55	56.0 14.69
% Post-Implantation Loss***	Mean SD	3.4 5.731	4.92 6.803	5.02 6.159	8.92 15.665
# of Non-Viable Fetuses/Animal	Mean SD	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0
Litter size**	Mean SD	13.1 1.91	12.2 1.2	11.4 2.46	11.4 3.39
Resorptions: Early + Late**	Mean SD	0.4 0.69	0.7 0.93	0.6 0.76	0.8 1.36
# of Early Resorptions**	Mean SD	0.3 0.45	0.7 0.93	0.6 0.76	0.8 1.36
# of Late Resorptions**	Mean SD	0.2 0.37	0.0 0.00	0.0 0.0	0.0 0.0

*Anova & Dunnett
**Kruskal-Wallis & Dunn: a = p < .05
***Anova & Dunnett (Arc SineSQRT100)

No test article-related maternal macroscopic findings were observed in the REL-1017 treated groups. All macroscopic findings in the treated animals were observed at a low incidence and/or were comparable to concurrent controls and were considered unrelated to treatment with REL-1017. *(Table 2)*

Table 3. Summary of Fetal Body Weight Values
Day(s): 20 Relative to Mating (Litter: A)

Sex: Female		0 mg/kg/day (vehicle)	10 mg/kg/day (REL-1017)	20 mg/kg/day (REL-1017)	40 mg/kg/day (REL-1017)
Fetal Weight Males* (g)	Mean SEM N	4.114 0.065 19	4.078 0.062 20	4.011 0.064 19	3.921 0.063 20
Fetal Weight Females* (g)	Mean SEM N	3.911 0.062 19	3.863 0.059 20	3.801 0.062 19	3.758 0.061 19
Mean Litter Fetal Weight* (g)	Mean SEM N	4.007 0.061 19	3.988 0.058 20	3.914 0.060 19	3.850 0.059 20

* = Ancova/Anova & Dunnett {Covariate: Litter size}, SEM = Standard Error of the Mean

Table 4. Summary of Individual Fetal External Observations

Exam Type: External		0 mg/kg/day (vehicle)	10 mg/kg/day (REL-1017)	20 mg/kg/day (REL-1017)	40 mg/kg/day (REL-1017)
# of Litters Examined # of Fetuses Examined		19 248	20 244	19 217	20 228
Forelimb (s) Entire, Short - Malformation	Fetuses N (%) Litters N (%) [f]*	0 (0.0) 0 (0.0)	0 (0.0) 0 (0.0)	0 (0.0) 0 (0.0)	1 (0.4) 1 (5.0)
Body Entire, Short – Malformation	Fetuses N (%) Litters N (%) [f]*	0 (0.0) 0 (0.0)	0 (0.0) 0 (0.0)	0 (0.0) 0 (0.0)	1 (0.4) 1 (5.0)
Skin, Dermal Hypoplasia - Variation	Fetuses N (%) Litters N (%) [f]*	0 (0.0) 0 (0.0)	0 (0.0) 0 (0.0)	0 (0.0) 0 (0.0)	1 (0.4) 1 (5.0)
Head Entire, Misshapen - Malformation	Fetuses N (%) Litters N (%) [f]*	0 (0.0) 0 (0.0)	0 (0.0) 0 (0.0)	0 (0.0) 0 (0.0)	1 (0.4) 1 (5.0)

*[f] = Fisher's Exact Test with Stepdown Sidak

Table 5. Summary of External Malformations and Developmental Variations

Exam Type: External			0 mg/kg/day (vehicle)	10 mg/kg/day (REL-1017)	20 mg/kg/day (REL-1017)	40 mg/kg/day (REL-1017)
# of Litters Examined # of Fetuses Examined			19 248	20 244	19 217	20 228
Malformation						
	# of Fetuses		0	0	0	1
	Group % of Fetuses		0.0	0.0	0.0	0.4
	# of Litters [f]	N	0	0	0	1
		%	0.0	0.0	0.0	5.0
	Litter % of Fetuses	Mean	0.00	0.00	0.00	0.36
Variation						
	# of Fetuses		0	0	0	1
	Group % of Fetuses		0.0	0.0	0.0	0.4
	# of Litters [f]	N	0	0	0	1
		%	0.0	0.0	0.0	5.0
	Litter % of Fetuses	Mean	0.00	0.00	0.00	0.42

*[f] = Fisher's Exact Test with Stepdown Sidak

No significant differences in fetal body weights were observed with REL-1017 at 10, 20, and 40 mg/kg/day as compared to control vehicle *(Table 3)*. No test article-related fetal external observations were observed at any REL-1017 dose level evaluated. No fetal external malformations or variations were observed in the control, 10, and 20 mg/kg/day groups *(Table 4 and Table 5)*. At 40 mg/kg/day, one fetus (animal number 4504, fetus 1) was observed with dermal hypoplasia (variation) and one fetus (animal number 4501, fetus 1) was observed with short forelimbs, short body, and misshapen head (all malformations) *(Table 4 and Table 5)*. The external malformations and variations at 40 mg/kg/day were observed at a low incidence and were within or comparable to recent historical control data and were considered unrelated to treatment with REL-1017.

Table 6. Summary of Individual Fetal Visceral Observations

Exam Type: Bouins		0 mg/kg/day (vehicle)	10 mg/kg/day (REL-1017)	20 mg/kg/day (REL-1017)	40 mg/kg/day (REL-1017)
# of Litters Examined # of Fetuses Examined		19 124	20 123	19 109	20 112
Thoracic cavity Thyroid, Smaller than normal - Variation	Fetuses N (%) Litters N (%) [f]*	1 (0.8) 1 (5.3)	0 (0.0) 0 (0.0)	0 (0.0) 0 (0.0)	1 (0.9) 1 (5.3)
Mouth Palate, Rugae irregular - Variation	Fetuses N (%) Litters N (%) [f]*	0 (0.0) 0 (0.0)	0 (0.0) 0 (0.0)	0 (0.0) 0 (0.0)	2 (1.8) 1 (5.3)

*[f] = Fisher's Exact Test with Stepdown Sidak

Table 7. Summary of Visceral Malformations and Development Variations

Exam Type: Bouins			0 mg/kg/day (vehicle)	10 mg/kg/day (REL-1017)	20 mg/kg/day (REL-1017)	40 mg/kg/day (REL-1017)
# of Litters Examined # of Fetuses Examined			19 124	20 123	19 109	20 112
Malformation						
	# of Fetuses		0	0	0	0
	Group % of Fetuses		0.0	0.0	0.0	0.0
	# of Litters [f]*	N	0	0	0	0
		%	0.0	0.0	0.0	0.0
	Litter % of Fetuses	Mean	0.00	0.00	0.00	0.00
Variation						
	# of Fetuses		1	0	0	3
	Group % of Fetuses		0.8	0.0	0.0	2.7
	# of Litters [f]*	N	1	0	0	2
		%	5.3	0.0	0.0	10.5
	Litter % of Fetuses	Mean	0.88	0.00	0.00	6.02

*[f] = Fisher's Exact Test with Stepdown Sidak

No test article-related fetal visceral observations were observed at REL-1017 dose level evaluated. No fetal visceral malformations or variations were observed in the 10 and 20 mg/kg/day groups and no fetal malformations were observed in the 40 mg/kg/day group.

Table 8. Summary of Skeletal Malformations and Developmental Variations

Exam Type: Skeletal			0 mg/kg/day (vehicle)	10 mg/kg/day (REL-1017)	20 mg/kg/day (REL-1017)	40 mg/kg/day (REL-1017)
# of Litters Examined # of Fetuses Examined			19 125	20 121	19 108	20 116
Malformation						
	# of Fetuses		0	0	0	3
	Group % of Fetuses		0.0	0.0	0.0	2.6
	# of Litters [f]*	N	0	0	0	3
		%	0.0	0.0	0.0	15.0
	Litter % of Fetuses	Mean	0.00	0.00	0.00	3.10
Variation						
	# of Fetuses		61	61	51	56
	Group % of Fetuses		48.8	50.4	47.2	48.3
	# of Litters [f]*	N	17	18	17	18
		%	89.5	90.0	89.5	90.0
	Litter % of Fetuses	Mean	46.64	48.48	46.59	45.67

*[f] = Fisher's Exact Test with Stepdown Sidak

No test article-related fetal skeletal observations were observed at any REL-1017 dose level evaluated. The skeletal malformations and variations observed in the treated groups were either observed at a comparable incidence to concurrent controls and/or recent historical control data, and/or at a low incidence and were considered unrelated to treatment with REL-1017.

CONCLUSIONS

In conclusion, in this embryo fetal development study, there was no evidence of REL-1017 (esmethadone hydrochloride) related toxic effects on maternal health or on fetal development. Based upon these findings, the no-observed-adverse-effect level for both maternal and developmental toxicity was 40 mg/kg/day, the highest REL-1017 (esmethadone hydrochloride) dose evaluated. This dose corresponded to a maximum observed plasma concentration (Cmax) at gestational day 17 (GD 17 Cmax) = 738 ng/mL, to an AUC 0 - 24 hours at gestational day 17 = 9920 hr*ng/mL, and to an accumulation ratio of 1.62.

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