Original Article

Embryo-Fetal Developmental Toxicity Studies with Pregabalin in Mice and Rabbits

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Pregabalin was evaluated for potential developmental toxicity in mice and rabbits. Pregabalin was administered once daily by oral gavage to female albino mice (500, 1250, or 2500 mg/kg) and New Zealand White rabbits (250, 500, or 1250 mg/kg) during organogenesis (gestation day 6 through 15 [mice] or 6 through 20 [rabbits]). Fetuses were evaluated for viability, growth, and morphological development. Pregabalin administration to mice did not induce maternal or developmental toxicity at doses up to 2500 mg/kg, which was associated with a maternal plasma exposure ($AUC_{0–24}$) of 3790 μg•hr/ml, ≥30 times the expected human exposure at the maximum recommended daily dose (MRD; 600 mg/day). In rabbits, treatment-related clinical signs occurred at all doses ($AUC_{0–24}$ of 1397, 2023, and 4803 μg•hr/ml at 250, 500, and 1250 mg/kg, respectively). Maternal toxicity was evident at all doses and included ataxia, hypoactivity, and cool to touch. In addition, abortion and females euthanized moribund with total resorption occurred at 1250 mg/kg. There were no treatment-related malformations at any dose. At 1250 mg/kg, compared with study and historical controls, the percentage of fetuses with retarded ossification was significantly increased and the mean number of ossification sites was decreased, which correlated with decreased fetal and placental weights, consistent with in utero growth retardation. Therefore, the no-effect dose for developmental toxicity in rabbits was 500 mg/kg, which produced systemic exposure approximately 16-times human exposure at the MRD. These findings indicate that pregabalin, at the highest dose tested,was not teratogenic in mice or rabbits. *Birth Defects Res (Part B)* 107:85–93, 2016. © 2016 The Authors Birth Defects Research Published by Wiley Periodicals, Inc.

Key words: ***pregabalin; developmental toxicity; teratology; mice; rabbits***

INTRODUCTION

Pregabalin (Lyrica®; Pfizer, New York, NY) is an α2-δ subunit ligand of voltage-gated calcium channels in the brain and spinal cord. The binding to the α2-δ subunit is believed to mediate its nociceptive, anxiolytic, and anticonvulsant actions (Stahl et al., 2013). Pregabalin is available in over 100 countries. In the United States, pregabalin is indicated as adjunctive treatment for partial seizures, for pain associated with fibromyalgia, postherpetic neuralgia, diabetic peripheral neuropathy, and spinal cord injury. It is approved in Europe as adjunctive treatment for partial seizures, central, and peripheral neuropathic pain, and generalized anxiety disorder (Pfizer, 2013, 2015).

As per the International Conference on Harmonisation guidelines (ICH, 1993), a series of nonclinical developmental toxicity studies were conducted prior to registering pregabalin for human use. Results from four developmental toxicity studies conducted in rats are provided in a companion paper (Morse et al., 2016). Briefly, pregabalin was shown not to be teratogenic when administered by oral gavage to Wistar rats during organogenesis at doses up to and including 2500 mg/kg/day. Pregabalin produced maternal toxicity (reduced body weight) and/or developmental toxicity (reduced viability and fetal body weight, increased skeletal variations). An increased incidence of fusion of the jugal bone and maxilla occurred in fetuses from females treated with ≥1250 mg/kg doses, and an increased incidence of fusion of the nasal sutures occurred at 2500 mg/kg. Based on historical and experimental data, fusions of the skull bones represented advanced closure of suturae and were classified as skeletal variations. Pregabalin was not teratogenic in rats under the conditions of these studies.

The mouse and rabbit were selected as additional species to evaluate the potential developmental toxicity of pregabalin. Both species are recommended in the U.S. Food and Drug Administration (FDA) guidelines and the ICH guidelines for reproduction studies (FDA, 1988; ICH, 1993) and have historical information for reference purposes when evaluating study findings. This paper presents results from two developmental toxicity studies

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(one in the mouse and one in the rabbit) conducted to assess the effects of pregabalin on pregnancy and embryo-fetal development when given once daily by oral gavage throughout the period of organogenesis.

METHODS

Both studies adhered to guidelines for animal welfare (National Institutes of Health, 1985/1996) and were conducted at Parke-Davis Pharmaceutical Research Division, Department of Pathology and Experimental Toxicology, Ann Arbor, MI, according to the U.S. FDA Good Laboratory Practice regulations (FDA, 1988) and by the Parke-Davis Research Animal Care and Use Committee.

Female albino mice [Crl:CD-1® (ICR)BR VAF/PLUS®; Charles River Laboratories, Inc., Portage, MI] and female New Zealand White rabbits [Hra:(NZW)SPF; Hazleton Research Products, Kalamazoo, MI] were used in the studies. Female mice were mated with mature males from the same strain and source, and the presence of a vaginal plug was designated gestation day (GD) 0. Female rabbits were superovulated by an intravenous injection of 50 IU human chorionic gonadotropin (hCG) approximately 3 weeks prior to artificial insemination. The females were artificially inseminated with semen from male rabbits from the same source. Following insemination, ovulation was induced by an intravenous injection of 100 IU hCG. The day of artificial insemination in rabbits was designated GD 0. On GD 0, mice were 11–12 weeks old and body weights were 24–31 g; rabbits were approximately 7 months old and body weights ranged from 2735–3494 g. During the study, all animals were housed individually. Food (mouse, Purina Certified Rodent Chow 5002; rabbit, Purina Certified High Fiber Chow 5325) was provided in accordance with standard laboratory practice and water was supplied ad libitum. Environmental conditions were set to 12/12 h light/dark cycle, temperature 68–78°F or 60–70°F for mice and rabbits, respectively, and relative humidity was 30–75%.

Dosing suspensions were prepared every 2 weeks (mouse study) or weekly (rabbit study) by suspending bulk pregabalin in aqueous 0.5% methylcellulose (MC). The volume of 25 ml/kg (mice) or 5 ml/kg (rabbits) was based on the most recent individual body weight.

Mated female mice were assigned randomly to treatment groups of 25 per group for the main study and additional mice (five in the vehicle control group and 25 in each pregabalin-treated group) were assigned to a toxicokinetic (TK) subgroup for plasma drug concentration analysis. Rabbits were randomly assigned by body weights to four groups of 24; the last four rabbits per group were assigned to a TK subgroup.

Doses of pregabalin (500, 1250, or 2500 mg/kg for mice or 250, 500, or 1250 mg/kg for rabbits) were administered daily by oral gavage on GD 6–15 (mice) or GD 6–20 (rabbits). Additional groups (mice, $n = 30$; rabbits, $n = 24$) received an equivalent dose of 0.5% MC to serve as vehicle controls.

In the mouse study, dose selection was based on previous studies in mice: an acute oral toxicity study with pregabalin and a teratology study with a structurally related compound (Pfizer, unpublished data). In the rabbit study, dose selection was based on results from an embryo-fetal study conducted in rats (data presented in companion paper) (Morse et al., 2016) and results of teratology studies in rats and rabbits with a structurally related compound (Pfizer, unpublished data).

All maternal females were observed daily for clinical signs of toxicity. In mice, body weight and food consumption were recorded pre-test and on GD 0, 6, 9, 12, 16, and 18. In rabbits, body weight was recorded pre-test and on GD 0, 6, 8, 10, 12, 14, 16, 18, 20, 21, 22, 24, 26, 28, and 30, and food consumption was recorded every other day from GD 0 through termination.

On presumed GD 11, blood samples from TK mice were collected from the thoracic cavity of control group at 4 h post-dose and from the pregabalin-treated group at approximately 0 (pre-dose), 1, 4, 7, and 12 h post-dose following CO_2 euthanasia (five mice per dose group and time point). On presumed GD 14, blood samples from TK rabbits were collected via the jugular vein from the control group at 4 h post-dose and at approximately 0 (pre-dose), 1, 2, 4, 6, and 12 h post-dose from the pregabalin-treated groups (four TK rabbits per dose group). All blood samples were collected in tubes containing sodium heparin. The blood was centrifuged, and the plasma was stored frozen until analyzed for pregabalin concentrations at the Pharmacokinetics and Drug Metabolism Department, Parke-Davis Pharmaceutical Research, Ann Arbor, MI, using a validated high-performance liquid chromatography procedure, originally developed for rat plasma and subsequently modified for rabbit and mouse plasma (Pfizer, unpublished data).

At scheduled euthanasia, mice were euthanized in a CO_2 atmosphere and rabbits were euthanized by an intravenous overdose of Surital®. The TK mice were euthanized prior to blood collection (GD 11), and the TK rabbits were euthanized the day following blood collection (GD 15) with no gross necropsy examination. The uterus of each TK animal was examined to determine pregnancy status. All surviving females were euthanized on GD 18 or GD 30 for mice and rabbits, respectively, and examined grossly: the number of corpora lutea, location and status of the implant site (live or dead fetus, early or late resorption), and sex and individual weight of each live fetus was recorded. The placenta of each live fetus was examined and weighed.

All term fetuses were euthanized and examined for external variations and malformations. Following external inspection, all fetuses were examined by fresh dissection for visceral variations and malformations and external/visceral observation data were recorded. Approximately two-thirds (mice) or all (rabbits) of the fetuses per litter were eviscerated, cleared, stained with Alizarin Red S, and examined for skeletal malformations and variations. The heads (sliced) of the remaining mice fetuses were fixed in 10% formalin and examined.

For purposes of evaluation, malformations were defined as developmental deviations that: (1) are gross structural changes, (2) may be incompatible with life, and (3) are generally rare in occurrence. Anatomic variations are structural alterations that occur infrequently but more often than malformations, and have no significant biological effect on body conformation, function, or general well-being. Skeletal variations are divided into the following categories: anatomic variations, ossification

Table 1
Mean Plasma Pregabalin Pharmacokinetic Values in Mice on GD 11

Plasma pregabalin parameters	Pregabalin dose (mg/kg/day), mean		
	500 (n = 3–4)[a]	1250 (n = 4–5)	2500 (n = 4–5)
C_{max} [μg/ml] ± SD	291 ± 22.3	640 ± 291	1310 ± 310
$AUC_{0–24}$ [μg•h/ml]	706	1680	3790

[a]Data excluded from non-pregnant animals.
$AUC_{0–24}$, area under the plasma pregabalin concentration-time curve from 0–24 h; C_{max}, maximum observed pregabalin concentration; GD, gestation day; SD, standard deviation.

retardation, and decreases in ossification sites. Evidence of teratogenicity is characterized by an increase in all of the following: (1) percent malformed offspring per litter, (2) number and percent of litters with malformed offspring, and (3) number of offspring or litters with a particular malformation that appears to increase with dose. A dose-related increase in the incidence of variations is considered an indication of developmental toxicity.

Statistical Analyses

In all cases, the class-wise significance level was allocated to each parameter proportionally by the inverse of the square root of the number of parameters in a class. Most quantitative reproductive parameters were analyzed using Tukey's sequential trend test using the rank-dose scale and rank-transformed data, one- or two-tailed, at the 5% class-wise significance level. This trend test is equivalent to sequential application of Kruskal–Wallis one-way analysis of variance by ranks, with the treatment effects being evaluated by dose-trend tests that have contrast coefficients for equally spaced (ranked) treatment groups. If the high-dose linear trend test was not statistically significant, a test of trend reversal was performed at the 1% class-wise significance level. If the test of trend reversal test was statistically significant, then Dunnett's test was used to compare the treated groups with the vehicle control.

Malformation, variation, and skeletal ossification parameters were evaluated by applying weighted nonparametric Tukey's sequential trend test to the vehicle control and the pregabalin-treated groups at the 5% class-wise significance level. The value of the weight variable was the number of fetuses examined. The analyses of percent litters with malformed fetuses or fetuses with variations were performed by sequential application of a weighted Cochran-Armitage test for linear trend in proportions at the 5% class-wise significance level. To assure that the linear dose-response relationship was realistic, a nonlinearity test was performed at the 1% class-wise significance level, two-tailed. If the high-dose Cochran-Armitage test was not statistically significant, a nonlinearity test was performed at the 1% class-wise significance level. If the nonlinearity test was statistically significant, then Fisher's exact test, one-tailed, at the 5% class-wise significance

Table 2
Maternal Reproductive and Litter Parameters in Mice

Parameter	Vehicle/control	Pregabalin dose (mg/kg/day)		
		500	1250	2500
Total mated females, n	25	25	25	25
Total pregnant females, n	22	23	22	23
Total females with viable fetuses, n	22	22	20[a]	23
Total females delivered early, n	0	0	2	0
Total females with resorptions only, n	0	1	0	0
No. of corpora lutea, mean ± SE	14.0 ± 0.45	12.0 ± 0.48	12.8 ± 0.47	13.2 ± 0.36
No. of implantation sites, mean ± SE	13.0 ± 0.39	11.4 ± 0.57	12.4 ± 0.36	12.3 ± 0.30
No. of resorptions, mean ± SE	1.0 ± 0.23	1.1 ± 0.61	1.0 ± 0.23	0.8 ± 0.21
No. of live fetuses per litter, mean ± SE	12.0 ± 0.42	10.2 ± 0.71	11.3 ± 0.38	11.4 ± 0.39
No. of dead fetuses per litter, mean ± SE	0.0 ± 0.05	0.1 ± 0.09	0.1 ± 0.07	0.1 ± 0.07
Preimplantation loss, %[b]	6.8 ± 1.86	7.9 ± 3.84	3.0 ± 1.14	6.0 ± 1.75
Postimplantation loss, %[c]	7.7 ± 1.81	9.4 ± 4.36	8.8 ± 1.62	7.9 ± 1.84
Sex ratio, % male	51.4 ± 2.71	48.4 ± 3.68	49.8 ± 4.47	52.4 ± 3.38
Fetal body weight (g), mean ± SE				
Males	1.06 ± 0.047	1.16 ± 0.035	1.07 ± 0.054	1.04 ± 0.038
Females	1.01 ± 0.047	1.11 ± 0.036	1.03 ± 0.052	1.00 ± 0.039
Placenta weight (g), mean ± SE	0.089 ± 0.0025	0.093 ± 0.0028	0.090 ± 0.0022	0.097 ± 0.0028

[a]Excludes animals that delivered viable litters prior to GD 18.
[b]Preimplantation loss per litter = [(corpora lutea − implantation sites)/corpora lutea] × 100.
[c]Postimplantation loss per litter = [(implantation sites − viable fetuses)/implantation sites] × 100.
GD, gestation day; SE, standard error.

Table 3
Incidence of Malformations and Variations in Term Fetuses from Pregnant Mice

Parameter (No. of fetuses/litters)[a]	Vehicle/control	Pregabalin dose (mg/kg/day)		
		500	1250	2500
Malformations				
External and visceral findings				
No. examined	264/22	237/22	247/22	265/23
No. with malformations	2/2	0/0	0/0	4/2
Exencephaly	–	–	–	1/1
Ablepharia	–	–	–	3/1
Cleft face	1/1	–	–	–
Cleft palate	1/1	–	–	–
Diaphragmatic hernia	–	–	–	1/1
Skeletal findings				
No. examined	184/22	164/22	158/20	184/23
No. with malformations	4/4	0/0	2/2	5/4
Skull bone(s)				
Agenesis	–	–	–	1/1
Malformed	–	–	–	1/1
Sternum				
Fused	2/2	–	2/2	3/2
Vertebrae				
Malformed	1/1	–	–	1/1
One less presacral	1/1	–	–	–
Variations				
Skeletal findings				
No. examined	184/22	164/22	158/20	184/23
Skull bone(s)				
Extra site of ossification	25/12	22/9	31/14	26/14
Hypoplastic (retardation)	–	3/1	2/2	3/2
Unossified bone(s) (retardation)	–	1/1	1/1	–
Ribs				
Extra well-formed lumbar	48/17	38/15	39/13	43/17
Extra rudimentary lumbar	58/20	55/15	40/15	52/20
Extra well-formed cervical	–	–	–	1/1
Extra rudimentary cervical	27/13	31/12	27/12	30/14
Sternebrae				
Asymmetric form	6/5	10/7	4/4	3/2
Extra ossification site	3/3	2/2	3/2	3/3
Focal fusion	–	1/1	–	–
Limbs				
Calcaneus (advanced)	121/21	115/19	113/18	88/19
Talus (advanced)	4/4	6/3	2/1	–
Vertebrae				
Hypoplastic (retardation)	2/1	–	–	–
Digits				
Mid phalanges (advanced)	182/22	162/21	149/19	182/23

No external/visceral anatomic variations were observed.
[a]Fetuses may have more than one malformation/variation.
–: not observed.

level, was used to compare the treated groups with the vehicle control.

RESULTS

Mice

There were no treatment-related clinical observations or deaths in this study. Maternal body weight gain and food consumption were not adversely affected during treatment (Pfizer, unpublished data). On GD 11, following repeated daily oral administration of pregabalin, mean maximal concentrations of pregabalin (C_{max}) and area under the plasma concentration-time curve ($AUC_{0–24}$) increased with increasing dose (Table 1). The highest dose (2500 mg/kg) was associated with a plasma exposure ≥30 times the expected human exposure (123 μg•h/mL) at the maximum recommended daily dose (MRD) of 600 mg/day (Pfizer, 2013).

No treatment-related macroscopic findings were observed at maternal necropsy. The mean number of corpora lutea, implantation sites, pre- and postimplantation loss, fetal sex ratio, fetal weight, and placental weight were comparable among pregabalin-treated and control groups (Table 2).

The incidence of fetuses and litters with malformations or variations were comparable between

Table 4
Maternal Body Weight Gain and Food Consumption in Rabbits

		Pregabalin dose (mg/kg/day), (*n*) mean ± SE		
	Vehicle/control	250	500	1250
Weight gain (g)				
GD 6–14	(18) 183.0 ± 14.0	(19) 208.8 ± 16.5	(20) 201.7 ± 13.5	(20) 210.1 ± 25.8
GD 14–21	(15) 106.2 ± 15.1	(16) 137.7 ± 22.8	(16) 152.1 ± 14.8	(15) 138.7 ± 14.2
GD 6–21	(15) 278.9 ± 14.3	(16) 349.5 ± 17.6*	(16) 348.9 ± 12.0*	(15) 354.7 ± 19.5*
GD 21–30	(15) 147.1 ± 20.9	(16) 81.1 ± 24.6	(16) 33.0 ± 25.7*	(15) 35.2 ± 21.9*
Food consumption (g)				
GD 6–14	(18) 1666.6 ± 53.5	(19) 1727.0 ± 55.7	(20) 1727.7 ± 45.5	(19) 1587.5 ± 93.1
GD 14–20	(15) 1252.7 ± 46.0	(16) 1367.7 ± 49.4	(16) 1368.7 ± 55.7	(14) 1396.6 ± 31.2
GD 6–20	(15) 2900.3 ± 98.9	(16) 3101.6 ± 96.2	(16) 3094.1 ± 79.5	(13) 3010.7 ± 68.9
GD 20–30	(15) 1846.7 ± 71.0	(15) 1735.1 ± 89.5	(16) 1782.3 ± 79.1	(14) 1635.0 ± 58.1

*$p < 0.0250$, different from vehicle control for trend test.
GD, gestation day; SE, standard error.

pregabalin-treated groups and the vehicle control group (Table 3). Ossification parameters were also comparable among groups (Pfizer, unpublished data).

Rabbits

Treatment-related clinical signs of maternal toxicity were observed in the pregabalin 250, 500, and 1250 mg/kg dose groups and included ataxia (7, 13, and 15 animals/group at 250, 500, and 1250 mg/kg); hypoactivity (10, 23, 24 animals/group at 250, 500, and 1250 mg/kg); and cool to touch (5, 9, 11 animals/group at 250, 500, and 1250 mg/kg). These findings were not observed in the control group and appeared to be generally dose-related (based on number of animals affected

Table 5
Mean Plasma Pregabalin Pharmacokinetic Values in Rabbits on GD 14

	Pregabalin dose (mg/kg/day), mean ± SD		
Plasma pregabalin parameters	250 ($n = 3$)	500 ($n = 4$)	1250 ($n = 4$)
C_{max} [μg/ml]	236 ± 26.2	266 ± 32.8	392 ± 16.5
T_{max} [h]	1.0 ± 0.0	1.8 ± 0.5	2.5 ± 1.0
$AUC_{0–24}$ [μg•h/ml]	1397 ± 198.6	2023 ± 303.2	4803 ± 863.7

$AUC_{0–24}$, area under the plasma pregabalin concentration-time curve from 0–24 h; C_{max}, maximum observed pregabalin concentration; GD, gestation day; SD, standard deviation; T_{max}, time of C_{max}.

Table 6
Reproductive and Litter Parameters in Rabbits

		Pregabalin dose (mg/kg/day)		
Parameter	Vehicle/control	250	500	1250
Total mated females, *n*	20	20	20	20
Total pregnant females, *n*	15	16	16	17
Total females aborted, *n*	0	0	0	1
Total females euthanized moribund, *n*	0	0	0	1[a]
Total females with resorptions only, *n*	0	0	0	1[a]
Total females delivered early, *n*	0	0	0	0
Total females with viable fetuses, *n*	15	16	16	15
No. of corpora lutea, mean ± SE	6.7 ± 0.49	7.7 ± 0.47	7.8 ± 0.48	7.3 ± 0.48
No. of implantation sites, mean ± SE	6.0 ± 0.57	6.4 ± 0.62	6.1 ± 0.56	6.9 ± 0.51
No. of resorptions, mean ± SE	0.5 ± 0.19	0.4 ± 0.15	0.9 ± 0.31	0.3 ± 0.16
No. of live fetuses per litter, mean ± SE	5.5 ± 0.64	6.0 ± 0.64	5.3 ± 0.55	6.5 ± 0.40
No. of dead fetuses per litter, mean ± SE	0.0 ± 0.0	0.1 ± 0.06	0.0 ± 0.0	0.1 ± 0.07
Preimplantation loss, %[b]	13.2 ± 5.63	17.4 ± 6.09	21.2 ± 5.31	6.9 ± 3.15
Postimplantation loss, %[c]	13.1 ± 5.07	7.5 ± 3.10	13.1 ± 4.51	4.6 ± 1.77
Sex ratio, % male ± SE	45.8 ± 7.72	45.1 ± 5.88	48.4 ± 6.73	46.1 ± 5.37
Fetal body weight (g), mean ± SE				
Males	56.4 ± 1.73	50.7 ± 1.46	53.3 ± 1.65	49.1 ± 1.22*
Females	54.3 ± 1.39	49.8 ± 1.26	50.9 ± 1.76	44.8 ± 1.23*
Placenta weight (g), mean ± SE	3.85 ± 0.242	3.56 ± 0.157	3.95 ± 0.220	3.28 ± 0.102

[a]Events occurred in same individual, excluded from statistical analysis.
[b]Preimplantation loss per litter = [(corpora lutea − implantation sites)/corpora lutea] × 100.
[c]Postimplantation loss per litter = [(implantation sites − viable fetuses)/implantation sites] × 100.
*$p < 0.0204$, different from vehicle control for trend test.
SE, standard error.

Table 7
Incidence of Malformations in Term Fetuses from Pregnant Rabbits

Parameter	Vehicle/control	Pregabalin dose (mg/kg/day)		
		250	500	1250
External and visceral findings				
Percent fetuses per litter with malformations ± SE	1.7 ± 1.67	1.3 ± 1.25	0.0 ± 0.0	3.9 ± 2.21
Percent litters with malformations	6.7	6.3	0.0	20.0
No. of fetuses/litters[a]				
No. examined	82/15	97/16	84/16	98/15
Total no. of external and visceral malformations	1/1	1/1	0/0	4/3
Eye – unilateral ablepharia	–	1/1	–	–
Head – domed-shaped with dilated ventricles	–	1/1	–	–
Jaw – agnathia	–	1/1	–	–
Mouth – astomia	–	1/1	–	–
Palate – high arch	–	1/1	–	–
Tongue – aglossia	–	1/1	–	–
Digits – brachydactyly/ectrodactyly	–	1/1	–	–
Tail – short	–	–	–	1/1
Aorta – bulbous	–	1/1	–	–
Diaphragm – hernia	–	–	–	1/1
Gallbladder – agenesis	1/1	–	–	2/1
Heart – interventricular septal defect	–	1/1	–	–
Vessel – thrombus	–	–	–	1/1
Skeletal findings				
Percent fetuses per litter with malformations ± SE	0.0 ± 0.00	0.9 ± 0.89	0.0 ± 0.00	4.4 ± 2.19*
Percent litters with malformations	0.0	6.3	0.0	26.7
No. of fetuses/litters[1]				
No. examined	82/15	96/16	84/16	97/15
Total no. of skeletal malformations	0/0	1/1	0/0	5/4
Ribs – fused	–	1/1	–	–
Sternebrae – fused	–	–	–	2/2
Vertebrae				
Agenesis	–	–	–	1/1
Fused	–	–	–	1/1
Malformed	–	–	–	1/1
One less presacral	–	–	–	1/1

[a]Fetuses may have more than one malformation. –: not observed.
*$p < 0.025$, different from vehicle control for trend test, one-tailed.
SE, standard error.

and incidence). The majority of these signs were observed on GD 6, 7, or 8. One treatment-related death occurred in the pregabalin 1250 mg/kg dose group. The rabbit was euthanized moribund on GD 21 due to antemortem clinical signs including hypoactivity, cool to touch, emaciation, and reduced/soft feces. Maternal body weight gain in the pregabalin-treated groups was variable over the course of the study (Table 4). Compared with the controls, mean maternal body weight gain was 25% (250 and 500 mg/kg) to 27% (1250 mg/kg) significantly greater in all pregabalin-treated groups during the overall treatment period (GD 6–21). Conversely, in the 500 and 1250 mg/kg dose groups, weight gain was significantly lower than controls by 76% in the posttreatment period. Although not statistically significant, body weight gain was less than control by 45% at 250 mg/kg during the posttreatment period. The decrease in weight gain during the posttreatment period can be attributed to the reduced food consumption observed from GD 22–24 in the pregabalin-treated groups (39, 49, and 61% less than controls at 250, 500, and 1250 mg/kg, respectively). Food consumption was comparable between groups during all other intervals.

On GD 14, the mean $AUC_{0–24}$ and C_{max} values increased with increasing dose (Table 5). The 500 mg/kg dose was associated with a plasma exposure approximately 16-times the expected human exposure at the MRD.

Maternal necropsy revealed no treatment-related macroscopic findings. Two treatment-related effects on maternal reproductive parameters were observed in the 1250 mg/kg dose group: one rabbit aborted on GD 20 and the rabbit that was euthanized moribund on GD 21 had total resorption. Both rabbits lost body weight from GD 8 onwards; thus the abortion and total resorption may be secondary to the maternal body weight change. No other treatment-related effects were observed on maternal reproductive parameters, including mean numbers of corpora lutea, implantation sites, live and dead fetuses, litter size, and pre- and postimplantation losses (Table 6). In the 1250 mg/kg dose group, mean fetal body weight was significantly reduced in male (13%) and female fetuses (17%) compared with controls. Although not statistically significant, placental weight at 1250 mg/kg was less than control by 15% correlating with reduced fetal weights at that dose. There were no treatment-related effects on fetal sex ratio or survival at term.

Table 8
Incidence of Variations in Term Fetuses from Pregnant Rabbits

Parameter (No. of fetuses/litters)[a]	Vehicle/control	Pregabalin dose (mg/kg/day)		
		250	500	1250
External/visceral findings				
Percent fetuses per litter with variations ± SE	2.1 ± 1.41	3.9 ± 3.17	8.5 ± 4.37	15.8 ± 4.37*
Percent litters with variations	13.3	12.5	25.0	60.0*
No. of fetuses/litters				
No. examined	82/15	97/16	84/16	98/15
Gallbladder				
Bilobed	1/1	–	–	–
Discoloration	–	–	–	1/1
Decreased size	–	–	1/1	5/3
Lung				
Azygous lobe absent	1/1	–	4/1	3/3
Vessel				
Left carotid arising from innominate	–	4/2	3/2	6/4
Anatomic skeletal findings				
Percent fetuses per litter with variations ± SE	85.2 ± 5.80	68.4 ± 5.79	78.3 ± 7.81	74.3 ± 5.65
Percent litters with variations	100.0	100.0	93.8	100.0
No. of fetuses/litters				
No. examined	82/15	96/16	84/16	97/15
Limbs				
Epiphysis of femur (advanced)	11/7	4/1	7/5	4/2
Ribs				
Short 12th	–	–	–	1/1
Extra well-formed lumbar	50/13	47/16	55/14	50/13
Extra rudimentary lumbar	29/12	28/12	20/12	26/12
Skull bone				
Bent	1/1	6/5	2/2	7/6
Sternebrae				
Epiphysis (advanced)	–	–	2/2	–
Extra ossification site	–	2/2	3/2	1/1
Focal fusion	–	–	2/2	–
Asymmetric form	–	–	2/2	1/1
Vertebrae				
Focal fusion	–	–	–	1/1
Extra presacral	17/7	16/10	21/19	28/11
Misshapen centra	–	–	–	1/1
Ossification retardations				
Percent fetuses per litter with retardations ± SE	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	3.2 ± 2.15**
Percent litters with retardations	0.0	0.0	0.0	13.3
No. of fetuses/litters				
Digits				
Unossified	–	–	–	4/2

[a]Fetuses may have more than one variation.
–: not observed.
*$p < 0.0354$, different from vehicle control for trend test, one-tailed.
**$p < 0.025$, different from vehicle control for trend test, one-tailed.
SE, standard error.

The percentage of fetuses per litter with skeletal malformations, external/visceral variations, and skeletal ossification retardations was statistically significantly increased at pregabalin 1250 mg/kg compared with controls (Tables 7 and 8). The percentage of litters with external/visceral variations was statistically significantly increased at pregabalin 1250 mg/kg compared with controls (Table 8). The incidence of external/visceral and skeletal variations (with the exception of ossification retardation) and malformations in fetuses and litters from pregabalin-treated rabbits was within the range observed in historical control data (0–6.9% fetuses external/visceral malformation/litter, 0–5.0% fetuses with a skeletal malformation/litter, 5.9–43% external/visceral variations/litter, and 63.5–85.7% fetuses with skeletal variations/litter) (Pfizer, unpublished data) and therefore not considered indicative of developmental toxicity or teratogenicity.

The percentage of fetuses per litter with ossification retardation, namely unossified digits, was significantly increased at 1250 mg/kg compared with controls (Table 8). The mean number of ossification sites per litter (olecranon, tuberosities of the humerus, and epiphyses of the hindlimbs) was significantly decreased at 1250 mg/kg compared with controls (Table 9), and values were outside the historical control range (Pfizer, unpublished data).

Table 9
Ossification Centers of Fetuses from New Zealand White Rabbits

Ossification center (mean ± SE of No. ossified per litter)	Vehicle/control	Pregabalin dose (mg/kg/day) 250	500	1250
No. of fetuses examined	15	16	16	15
Sternebrae	5.9 ± 0.03	6.0 ± 0.00	6.0 ± 0.00	6.0 ± 0.02
Olecranon	1.3 ± 0.12	0.6 ± 0.14*	0.6 ± 0.14*	0.2 ± 0.07*
Tuberosities of humerus	4.0 ± 0.01	3.8 ± 0.07	3.8 ± 0.07	3.3 ± 0.24*
Forelimb – epiphyses	2.0 ± 0.00	2.0 ± 0.00	2.0 ± 0.00	2.0 ± 0.02*
Hindlimb – epiphyses	3.9 ± 0.04	3.9 ± 0.06	3.9 ± 0.08	3.2 ± 0.23*

*$p < 0.0224$, compared with vehicle control for trend test.
SE, standard error.

The differences in ossification parameters observed at 1250 mg/kg, correlated with decreased fetal and placental weights, were considered evidence of developmental toxicity.

DISCUSSION

In mice, daily treatment with pregabalin 500, 1250, or 2500 mg/kg did not induce maternal or developmental toxicity at the highest dose evaluated of 2500 mg/kg with a maternal C_{max} of 1310 μg/ml and an $AUC_{0–24}$ of 3790 μg•hr/ml (≥30-times the expected human exposure at the MRD). The incidence and percentage of fetuses and litters with malformations or variations was comparable between treated and control groups.

In contrast to the present study findings, a teratogenicity study conducted in BALB/c mice found that intraperitoneally (IP) administered pregabalin 20, 40, and 80 mg/kg/day from GD 6 through 15, significantly increased the rate of fetal skeletal malformations compared with control animals ($p < 0.001$, $p < 0.05$, and $p < 0.05$, respectively) (Etemad et al., 2013). The highest rate of total malformations was observed in the lowest dose group (20 mg/kg/day; 13.87% fetuses with skeletal malformation [vertebral column deformity, limb deformity, and cranialfacial abnormalities]) making the relationship to treatment unclear. Also in contrast to orally administered pregabalin, pregabalin administered IP at 40 and 80 mg/kg/day caused maternal toxicity and decreased fetal viability; the percentage of fetal resorption was significantly increased compared with the control group. The contrast in study findings is unlikely due to exposure. Pregabalin is highly absorbed in mice after oral exposure; 94% of dose (Pfizer, unpublished data). Exposure is dose-proportional up to 2500 mg/kg (Table 1). Therefore, exposure at 2500 mg/kg/day (oral administration) should greatly exceed exposure at 80 mg/kg/day (IP administration).

Treatment of pregnant rabbits with pregabalin induced clinical signs and decreased weight gain posttreatment at all doses ($AUC_{0–24}$ of 1397, 2023, and 4803 μg•hr/ml at 250, 500, and 1250 mg/kg, respectively). The treatment-related clinical signs, including ataxia and hypoactivity, were similar to those observed in the embryo-fetal and skull developmental studies conducted in rats (Morse et al., 2016). Maternal body weight gain during the overall treatment period was greater than controls in all pregabalin-treated groups (Table 4). Fetal growth retardation at 1250 mg/kg, demonstrated by decreased fetal weight and reduced ossification, may have been related to the decrease in maternal body weight gain posttreatment (Chernoff et al., 2008). Although the majority of maternal parameters were unaffected by treatment, maternal toxicity was evident at 1250 mg/kg and included abortion and moribund euthanasia with total resorption in one animal each. These two animals had the greatest weight loss; thus the maternal toxicity may be partially due to the weight loss.

Developmental toxicity in rabbits, manifested as in utero growth retardation, was evident at 1250 mg/kg. Significant increases in the percentage of fetuses with ossification retardation and significant decreases in the mean number of ossification sites correlated with decreased fetal and placental weights at this dose. However, there was no evidence of teratogenicity noted in rabbits. The external and visceral variations and the skeletal malformations observed in this study are relatively common findings in this strain of rabbits and comparable with those found in the concurrent historical control database (Pfizer, unpublished data).

CONCLUSIONS

Pregabalin administered to mice and rabbits during organogenesis did not produce teratogenesis at systemic exposures of ≥30-times the expected human exposure at the MRD. There was no evidence of developmental toxicity in mice. Decreased fetal body weight and delayed ossification of a small number of skeletal structures, indicative of in utero growth retardation, was considered to be evidence of developmental toxicity in rabbits.

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REFERENCES

Chernoff N, Rogers EH, Gage MI, Francis BM. 2008. The relationship of maternal and fetal toxicity in developmental toxicology bioassays with notes on the biological significance of the "no observed adverse effect level." Reprod Toxicol 25(2):192–202.

Etemad L, Mohammad A, Mohammadpour AH, Vahdati Mashhadi N, Moallem, SA. 2013. Teratogenic effects of pregabalin in mice. Iran J Basic Med Sci 16(10):1065–1070.

FDA. 1988. Good laboratory practice regulations for nonclinical laboratory studies. Code of federal regulations (CFR) (April 1). Washington, DC: US Food and Drug Administration.

ICH. 1993. International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use. Detection of toxicity to reproduction for medicinal products. http://www.ich.org/home.html.

Morse DC, Henck JW, Bailey SA. 2016. Developmental toxicity studies with pregabalin in rats: Significance of alterations in skull bone morphology. Birth Defects Res Part B 107(2):94–107.

National Institutes of Health. 1985/1996. Guide for the care and use of laboratory animals. NIH Publication. Washington, DC: The National Academies Press.

Pfizer. 2013. LYRICA® US prescribing information. Pfizer.

Pfizer. 2015. LYRICA® EU summary of product characteristics. Pfizer.

Stahl SM, Porreca F, Taylor CP, Cheung R, Thorpe AJ, Clair A. 2013. The diverse therapeutic actions of pregabalin: is a single mechanism responsible for several pharmacological activities? Trends Pharmacol Sci 34(6):332–339.