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Effects of the Oral Oxytocin Receptor Antagonist Tocolytic OBE001 on Reproduction in Rats

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Abstract

Background: OBE001 is a novel, orally active nonpeptide oxytocin receptor antagonist under development for the treatment of preterm labor and improvement in embryo implantation and pregnancy rate in assisted reproductive technology (ART). The reproductive safety of OBE001 was evaluated in customized fertility embryonic development (FER)/early embryonic development (EED) and fetal development (FD) and pre/postnatal development (PPN) studies mimicking clinical exposure scenarios. **Methods:** Oral OBE001 was evaluated at doses of 37.5, 75, and 125 mg/kg/d in female rats during a FER/EED study (from premating to implantation) and throughout FD during a FD/PPN study. **Results:** No OBE001 effects were observed during the FER/EED study. The FD/PPN study did not result in adverse OBE001 effects in females allowed to litter, their offspring, and second-generation fetuses. Females at 125 mg/kg/d who underwent cesarean section before term had slight reductions in body weights and food consumption, and associated fetuses had slightly delayed ossification of skull bones, which was not adverse in the absence of effects on live offspring. **Conclusion:** OBE001 at up to 125 mg/kg/d had no effects on EED and no adverse effects on FD and postnatal development of rats. These results constitute an important step toward the development of OBE001 in preterm labor and ART indications.

Keywords

preterm labor, assisted reproductive technology, OBE001, reproductive safety, rat

Introduction

OBE001 is a novel, orally active, nonpeptide oxytocin receptor antagonist (OTRan) being developed for the treatment of preterm labor and to improve embryo implantation and pregnancy rate in the context of assisted reproductive technology (ART).[1,2]

Premature birth before 37 weeks of gestation is a major problem in obstetrics affecting about 10% of all births worldwide.[3] It is the largest cause of perinatal morbidity and mortality. Preterm increase in uterine activity is a common complication of pregnancy and accounts for many cases of preterm labor. Therapeutic agents aimed at stopping uterine contractions (tocolysis) are the cornerstone of pharmaceutical management of preterm labor. The peptide hormone oxytocin is a potent contractor of the human uterus, and antagonists of the oxytocin receptor have been shown to reduce spontaneous preterm uterine activity in animal models and pregnant women.[4-7]

In ART, it has been shown that uterine contractile activity is increased in in vitro fertilization patients at the time of embryo transfer (ET) as compared with a spontaneous menstrual cycle.[8] A negative correlation has been reported between uterine contraction on the day of ET and the rate of embryo implantation and clinical pregnancy, possibly due to the uterine contractions expelling embryos out of the uterine cavity.[9,10]

Therefore, it is hypothesized that reducing uterus contractions at the time of ET by the administration of an OTRan may increase embryo implantation rate and thus pregnancy rate in ART.[11]

Available data have shown that OBE001 at repeated doses of 600 mg was well tolerated by healthy postmenopausal women.[12] With regard to testing new therapeutics in women of childbearing potential, health authorities acknowledge a

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high level of concern for the exposure of an embryo or fetus before information is available concerning the potential benefits versus potential risks. Pregnant women can only be admitted to larger scale clinical trials once all relevant animal reproduction toxicity studies are conducted, safety data from human exposure in nonpregnant female subjects have been evaluated, and no potential safety concerns are identified.[13] To support the development of OBE001, translational research was needed to allow identification and assess potential hazards based on the anticipated clinical use, especially in relation to reproduction.[14] For the ART indication with an OBE001 treatment around the time of ET, the translational rat study covered daily treatment including embryo implantation in the rat uterus (day 6 of gestation). OBE001 may be used to treat preterm labor during the period from the limit of viability (24 weeks of pregnancy) until 37 weeks, and the fetal development (FD) phase begins in week 10 of pregnancy. The corresponding rat study covered the entire FD period, from day 15 of gestation until birth (day 20 of gestation).

The studies presented in this article were undertaken to investigate the effect of OBE001, when administered by oral gavage to adult rats, on early embryonic development (EED) or FD and pre/postnatal development (PPN) in relation to its intended use during early and late pregnancy (Figure 1).

Materials and Methods

Preclinical Studies

Both studies were performed in accordance with Good Laboratory Practice. The FD/PPN study was conducted at RBM S.p.A. (Colleretto Giacosa, Italy) between January and October 2006. The conduct and reporting of the FER/EED study and a review of the FD/PPN study data were contracted to Covance Laboratories Ltd (Harrogate, United Kingdom) and performed between January and June 2014.

Animals and Husbandry

All rats were of the Sprague Dawley strain (Charles River Laboratories Italia S.p.A., Calco, Italy and Harlan, Blackthorn, United Kingdom) and were approximately 9 to 12 weeks of age at the start of the study. Following an acclimation period, the rats were housed in groups of up to 4 (prepairing and postpairing), 1 female with 1 male (pairing) or individually (mated and littered females), in polycarbonate cages in rooms maintained at 22°C $\pm$ 2°C, relative humidity 55% $\pm$ 10%, and with a 12-hour light–dark cycle. Food (GLP 4 RF 25 top certificate, produced by Charles River Italia's feed licensee, Mucedola srl, Settimo Milanese, Italy or SQC Rat and Mouse Breeder Diet No 3, Expanded, Special Diets Services Ltd, Witham, United Kingdom) and tap water were available ad libitum. For mating, each female was housed with 1 untreated male of the same strain, and the females were checked daily for the presence of sperm in a vaginal lavage. The day that sperm were found in the vaginal lavage was considered to be day 0 of gestation. All animals were checked daily for changes in appearance, behavior, mortality, and evidence of abortion. Body weights and food consumption were measured throughout the studies including pretreatment, treatment, and posttreatment periods. Blood samples were collected from selected animals in both the FER/EED and the FD/PPN studies at predetermined time points to confirm plasma exposure of OBE001. OBE001 concentrations were determined by liquid chromatography coupled with tandem mass spectrometry using a validated method (ObsEva, data on file).

Test Article

Solutions of OBE001 in an aqueous Labrasol (25% and 50% for the FER/EED and the FD/PPN studies, respectively) vehicle were prepared as appropriate for stability data. Dosing solutions were administered by oral gavage at dosing volumes of 4 mL/kg. Control animals received vehicle only at equivalent dosing volumes. Individual dose volumes were adjusted on most recently recorded body weights. OBE001 solution concentrations were between 95% and 102% of the theoretical value for all concentrations.

The FER/EED Study in Rats

This study was conducted to assess the potential reproductive effects of OBE001 on the period prior to mating, mating, and implantation of the conceptus in the uterine wall when administered to female rats for 14 days prior to mating and during mating until day 6 of gestation, inclusive.

OBE001 was administered to 20 females per group at dosage levels of 37.5, 75, or 125 mg/kg/d. Vaginal smears were evaluated daily for stage of estrus during 14 days of treatment preceding mating and during the mating period. Mating was 1:1 with untreated males for a maximum of 14 days. All females were euthanized via isoflurane anesthesia, cervical dislocation, exsanguination, and cesarean sectioned on day 13 of gestation. The pregnancy status and numbers of live and dead embryos, resorptions (early and late), and implantation sites were determined. The ovaries were examined and the corpora lutea counted. Blood samples for toxicokinetic (TK) analysis were collected after 14 days of treatment.

Combined FD and PPN Toxicity Study in Rats

This study assessed the effect of OBE001 on FD, parturition, and morphological and functional development of the offspring when administered orally to pregnant rats during the fetal period of gestation.

OBE001 was administered to 40 females/group at dosage levels of 37.5, 75, or 125 mg/kg/d from days 15 to 20 of gestation. Additionally, groups of mated female rats were added to document exposure to OBE001 (TK cohort), and these animals were cesarean sectioned on day 20 of gestation, and plasma level determination of OBE001 was performed.

The females were divided into 2 subgroups: 15 females/group were used for the assessment of FD (FD cohort), cesarean sectioned on day 20 of gestation, and their fetuses were subjected to skeletal and visceral examinations. The pregnant uterus and individual placental and fetus weights, numbers of corpora lutea, implantations, resorptions (early and late), and dead and viable fetuses per sex were determined. Individual fetuses were assessed for external malformations, anomalies, and variants. Approximately one-half of the fetuses underwent visceral examination with Bouin fixed heads examined after coronal sectioning.[15,16] The remaining fetuses were examined for skeletal alterations.

In the second part of the study, 25 females/group were allowed to litter and their pups were followed until day 21 of lactation and observed for morphological and functional development (PPN cohort). The dams and pups were euthanized on day 21 of lactation except those pups subjected to behavioral tests and reproductive performance (1 pup/sex/litter). At birth and during the lactation period, all pups were individually observed for external abnormalities at birth, sex, live, and stillbirths. Mortality, pup weight, and developmental landmarks (appearance of fur, pinna detachment, eruption of incisors, eye opening, and surface righting reflex) were recorded throughout the lactation period. The pups left alive for behavioral tests and reproductive performance (F1 generation) were weighed weekly and checked daily for clinical signs including cleavage of the balanopreputial gland and vaginal opening. Behavioral tests to evaluate neuromotoric development were performed at 7 weeks after birth and comprised the inclined plane, the water Y-maze, and the open-field test. At 12 weeks of age, the F1 animals were mated. The animals were weighed regularly during mating and pregnancy. The females were cesarean sectioned on day 20 of gestation, gross necropsy was performed, and their fetuses were subjected to external and visceral examinations as described for the FD cohort. The males were killed when spermatozoa were found in the vaginal smear of the female with which they were mated, and gross necropsy was performed.

Statistical Methods

Quantitative variables were analyzed using either a parametric analysis of variance (ANOVA) followed by a pairwise comparison using Dunnett test or a nonparametric Kruskal-Wallis test followed by a pairwise comparison using the Wilcoxon rank-sum test. The pairwise methods were used only when the overall ANOVA F test or the Kruskal-Wallis test was significant at an α level of 5%. Choice between the parametric and the nonparametric methods was based on checks for certain parametric assumptions, which were carried out using the Bartlett or Levene test. Both tests were performed using a 5% significance level. Frequencies and quantitative variables transformed into a binary scale were analyzed using the Fisher exact test. A 1-sided lower tail test tested for a decreasing response with increasing dose, and a 1-sided upper tail test tested for an increasing response with increasing dose.

Table 1. OBE001 Fertility and Early Embryonic Development Study: Reproductive and Exposure Parameters.

Daily Dose, mg/kg	0 (Control)	37.5	75	125
Toxicokinetics: $AUC_{0\text{-}t}$ day 14 (ng/mL·h)	-	35 100	61 500	94 900
No. evaluated	20	20	20	20
Premating body weight, ga	199.1	201.3	197.4	198.2
Gestation body weight, gb	267.6	275.0	274.2	267.4
Premating food consumption, gc	14.7	15.7	15.4	15.6
Gestation food consumption, gd	63.2	63.6	64.7	62.4
Mean no. estrous cycles/length, days	2.2/4.3	2.2/4.3	2.0/4.6	2.2/4.5
Mating index, %	100	100	100	100
Fertility index, %	95	95	95	95
No. of pregnant females	19	19	19	19
No. aborted or with total resorption of litter	0	0	0	0
Mean no. corpora lutea	15.1	16.9	16.5	16.6
Mean no. implantations	13.1	15.2	15.6	14.4
Mean percent preimplantation loss	12.7	9.9	6.2	13.7
Mean no. live conceptuses	12.9	14.7	15.0	13.4
Mean no. resorptions (early)	0.2	0.4	0.6	1.1
Mean percent postimplantation loss	1.8	2.7	3.9	7.2

Abbreviations: AUC, area under the curve; -, no noteworthy findings.
[a]Prepairing, day 1.
[b]Gestation, day 13; n = 19.
[c]Prepairing, days 1 to 4.
[d]Gestation, days 10 to 13; n = 19.

To compare the mating distribution during the time allowed for mating, the log-rank test was applied. Unless otherwise noted, all other statistical tests were performed at a level of 5%. Where appropriate, the data are expressed as mean $\pm$ standard deviation (SD).

Results

The FER/EED Study in Rats

There were no unscheduled deaths during the study (Table 1). Clinical observations were generally unremarkable and showed no relationship to treatment with OBE001, and mouth rubbing and paddling were seen across all groups including control. Salivation was also seen occasionally in all treated groups. These postdosing observations are commonly seen in this strain of rat and were considered to be a sign of taste aversion rather than systemic toxicity. There were no effects on body weight gain, food intake, number of estrous cycles prior to mating, and mean estrous cycle length. All females showed positive signs of mating, with 19 of 20 being pregnant at necropsy in all groups. Mating index, fecundity index, and fertility index were all unaffected by treatment. There were no remarkable

macroscopic necropsy findings. The numbers of corpora lutea, the incidence of preimplantation and postimplantation loss, and the number of live embryos per litter were unaffected by treatment with OBE001. The percentage of postimplantation loss was higher than controls at 125 mg/kg/d; however, the values for the control group, and 37.5 and 75 mg/kg/d, were lower than the historical control data range (Covance, data on file), whereas the 125 mg/kg/d value remained within the historical range and was therefore considered not to be affected by treatment with OBE001. Overall, based on the lack of study findings, the no observable effect level (NOEL) for maternal and embryo–fetal toxicity for OBE001 was considered to be 125 mg/kg/d. Plasma exposures at study dose levels are presented in Table 1.

Combined FD/PPN Toxicity Study in Rats

Following the start of oral gavage dosing on day 15 of gestation, mortality was observed in all groups (2 control females: 1 from each of the FD and the PPN cohorts, 4 females of the PPN cohort at 75 mg/kg/d, and 6 females at 125 mg/kg/d: 3 PPN, 1 FD, and 2 TK animals; Tables 2 and 3 and Supplemental Table S1). In addition, dyspnea (labored breathing) after treatment was seen in 2 control females (1 of which died subsequently), in 2 females at 37.5 mg/kg/d, in 3 females at 75 mg/kg/d (2 of which died), and in 3 females at 125 mg/kg/d (1 of which died). Salivation after dosing was observed in all groups including the controls. Evaluation of the pathology data from the main study decedent animals showed that there was edema and inflammation seen in the trachea and alveoli, which was sometimes seen also in the bronchioles.

Before dosing, maternal mean body weight gains of the PPN cohort were similar to the controls. There was no body weight effect after the start of dosing at any dose level. Between days 16 and 18 of gestation, mean body weight gain at 125 mg/kg/d was slightly lower than the controls, which may indicate a slight effect of treatment but was seen to recover between days 18 and 20 of gestation (40.33 vs 32.55 g, respectively) and was therefore considered to be not adverse. There was no effect on mean body weight gain at 37.5 or 75 mg/kg/d.

For the FD cohort, variability in group mean body weight gain prior to dosing was seen compared to controls. For 1 day after commencement of dosing at 125 mg/kg/d, there was slight mean body weight loss. Subsequently, there was lower body weight gain than the controls, but this was less each day and between days 18 and 20 of gestation was similar to the low-dose group indicating recovery. Therefore, the effect on mean maternal body weight at 125 mg/kg/d was considered to be not adverse. At 75 mg/kg/d, there was no effect on mean body weight gain after 1 day of dosing, body weight gain was lower than the controls on days 16 to 18 of gestation, and immediately followed by higher body weight gain than the controls on days 18 to 20 of gestation. This may be a delayed response to test article administration but was transient and therefore considered to be not adverse. No effect on mean body weight gain was seen at 37.5 mg/kg/d, and the slightly lower body weight gain between days 18 and 20 of gestation was considered to be coincidental.

Food consumption before dosing was comparable to controls for both cohorts. At all dose levels, mean food consumption during gestation before and after the commencement of dosing was similar to or higher than the controls in the PPN subset.

For the FD subset, after the commencement of dosing on day 15 of gestation, mean food consumption was lower than the controls by 14% to 17% across treatment groups, although this was not in a dosage-related manner and statistical significance was not achieved. Mean food consumption at 125 mg/kg/d remained similarly low on days 16 and 18 of gestation, but there was evidence of recovery by day 20 of gestation (after 5 days of dosing) when mean food consumption was only 8% lower than the controls and therefore considered to be similar. Mean food consumption for females at 37.5 or 75 mg/kg/d was similar to the controls on days 16, 18, and 20 of gestation.

With regard to reproductive parameters such as parturition and litter survival, there were no findings of significance in the PPN subgroup (Table 2). The length of gestation was approximately 22 days and was similar across all the groups, and there was no effect on parturition. A single stillborn pup was recorded at 125 mg/kg/d. This female lost 26 g body weight between days 16 and 18 of gestation which, while not unique, may be associated with the death in utero of a single pup. There were no significant differences between the control and the treated groups on the number of live pups per female, the birth index, and the pup survival. Pup body weights of the treated dams were similar to or higher than those of the control group. The physical and sexual developmental parameters observed in pups belonging to the treated groups were also similar to those of the control group, and there were no OBE001 effects on the reproductive performance or pregnancy body weights of the F1 generation and no effects on the F2 generation at cesarian section. Necropsy and organ weight measurements of the F1 generation did not reveal differences between the treated animals and the control group.

Since OBE001 treatment did not commence until implantations were established and embryos were in the latter part of organogenesis, reproductive parameters of interest in the FD group were late resorptions and dead fetuses. There was 1 dead fetus in each of the groups at 75 or 125 mg/kg/d. One animal at 125 mg/kg/d, with a dead fetus, had a marked lower body weight gain during the dosing period (20 g). The female had a large litter with 18 implantations and 17 live fetuses, which were small but did not have malformations at necropsy, and it was considered that the single fetal death was related to poor maternal condition. One animal at 75 mg/kg/d had a body weight gain of 57 g and was therefore unremarkable (range: 35 to 95 g). This female had 15 implantations and 14 live fetuses at necropsy. The litter weight was comparable with the other litters in this group, and there were no malformations found at fetal examination. It was therefore considered that this finding was coincidental. The mean number of live fetuses per female in the groups that received OBE001 was lower than the

Table 2. OBE001 Combined FD and PPN Development Study: Reproductive and Exposure Parameters.[a]

Daily Dose, mg/kg	0 (Control)	37.5	75	125
F_0 females—TK subgroup	3	6	6	6
Toxicokinetics: AUC_{24h} day 20 (ng/mL·h)	-	26 359	68 559	100 289
F_0 females—FD subgroup (cesarian sectioned)	14	15	14	14
Mean body weight gain (percent difference from control; actual weight gain, g)				
GD 14-15 (predose)	4.31	+40.8 (6.07)	+27.6 (5.50)	+89.1 (8.15)
GD 15-16	4.69	+5.1 (4.93)	+8.1 (5.07)	−54 (−2.54)
GD 16-18	28.85	+10.2 (31.79)	−40.6 (17.14)	−26.9 (21.08)
GD 18-20	34.54	−13.5 (29.86)	+17.7 (40.64)	−12.5 (30.23)
No. pregnant	13	14	14	13
Mean no. corpora lutea	20.23	18.79	19.29	19.77
Mean no. implantations	16.54	15.29	14.93	14.62
Mean percent preimplantation loss	17.74	18.21	21.89	23.47
Litters				
No. litters evaluated	13	14	14	13
No. live fetuses/per female	15.77	14.64	14.36	14.23
Mean no. resorptions (late)	0.08	0.00	0.00	0.08
No. of litters with dead fetuses	0	0	1	1
Mean percent postimplantation loss	5.22	4.01	3.83	2.39
Mean fetal body weight, g	3.91	3.88	3.71[b]	3.65[c]
Mean placental weight, g	0.54	0.59	0.58	0.61
Fetal sex ratio (% males)	47.80	50.73	48.76	49.19
Fetal malformations (frequency; percent of litters)				
Gross external	1/205 (7.69%)	0/205 (0.00%)	0/201 (0.00%)	0/185 (0.00%)
Fetal anomalies (frequency; % of litters)				
Gross external	0/205 (0.00%)	0/205 (0.00%)	0/201 (0.00%)	1/185 (7.69%)
Visceral anomalies	3/100 (23.08%)	0/99 (0.00%)	0/101 (0.00%)	0/95 (0.00%)
Skeletal anomalies	26/104 (69.23%)	34/106 (100.00%)	54/100[d] (92.86%)	45/90[d] (100.00%)
F_0 females—PPN subgroup (allowed to litter)	24	25	22	22
Mean body weight gain (percent difference from control; actual weight gain, g)				
GD 14-15 (Predose)	8.83 g	−15.5 (7.46)	−15.1 (7.50)	−5.1 (8.38)
GD 15-16	3.83 g	+75.2 (6.71)	+112.3 (8.13)	+12.0 (4.29)
GD 16-18	19.91 g	+45.1 (28.88[c])	+31.0 (26.09)	−10.4 (17.83)
GD 18-20	32.55 g	−2.1 (31.88)	−4.5 (31.10)	+23.9 (40.33)
No. delivered	22	24	21	21
Lactation body weight, ge	339.18	346.21	333.71	359.10[c]
Lactation food consumption (g/animal/d)f	69.11	69.02	70.36	73.28
Mean duration of gestation, days	22.09	22.29	22.33	22.10
Abnormal parturition	-	-	-	-
F_1 litters (preweaning)				
No. litters evaluated	22	24	21	21
Mean no. implantations	15.64	15.08	15.48	16.24
Mean no. liveborn pups/litter	14.82/22	13.83/24	14.14/21	14.67/21
Mean no. stillborn pups/litter	0/0	0/0	0/0	0.05/1
No. litters with stillborn pups	0	0	0	1
Postnatal survival to day 4, %	94.10	93.30	98.98	96.27
Postnatal survival to weaning, %	99.10	93.57	99.40	99.04
No. total litter losses	1	1	0	0
Pup body weights on day 0, g	6.13	6.59	6.75	6.19
Change in pup body weights, gg	36.00	42.20	39.89	38.45
Pup sex ratios (% males)	50.31	51.20	46.13	51.95
Pup clinical signs	-	-	-	-
Pup necropsy observations	-	-	-	-

Abbreviations: ANOVA, analysis of variance; AUC, area under the curve; FD, fetal development; GD, gestational day; PPN, pre/postnatal development; TK, toxicokinetic; -, no noteworthy findings.

[a]Postweaning parameters are presented in Supplemental Table S1. Statistical significance by ANOVA with Dunnett test or Kruskal–Wallis nonparametric ANOVA with Mann–Whitney *U* test: [b]$P < .01$, [c]$P < .05$, [d]$P < .001$.

[e]Day 21.

[f]Day 14.

[g]From birth to weaning.

Table 3. OBE001 Combined Fetal and Pre/Postnatal Development Study: Incidence of Specific Fetal Observations.[a]

Daily Dose, mg/kg	0 (Control)	37.5	75	125	Trend Test
No. examined fetuses	104	106	100	90	
Skeletal anomalies (frequency; %)					
Cranium					
Interparietal, incomplete ossification	20; 19.23%	25; 23.58%	47[c]; 47.00%	41[c]; 45.56%	.0001
Parietals, incomplete ossification	4; 3.85%	7; 6.60%	26[c]; 26.00%	20[c]; 22.22%	.0001
Supraoccipital, incomplete ossification	12; 11.54%	18; 16.98%	35[c]; 35.00%	21[c]; 23.33%	.0027
Vertebrae					
11th thoracic centrum, butterfly	1; 0.96%	1; 0.94%	2; 2.00%	4; 4.44%	.0338
12th thoracic centrum, butterfly	5; 4.81%	1; 0.94%	5; 5.00%	0[b]; 0.00%	>.05
Sternum					
5th sternebra, bipartite	2; 1.92%	0; 0.00%	0; 0.00%	0; 0.00%	.0384
5th sternebra, hemisternebra	6; 5.77%	3; 2.83%	10; 10.00%	9; 10.00%	.0438
5th sternebra, unossified	13; 12.50%	4[b]; 3.77%	12; 12.00%	17; 18.89%	.0266
6th sternebra, unossified	12; 11.54%	4[b]; 3.77%	10; 10.00%	7; 7.78%	>.05

[a]Statistical significance by Fisher exact test: [b]$P < .05$, [c]$P < .001$.

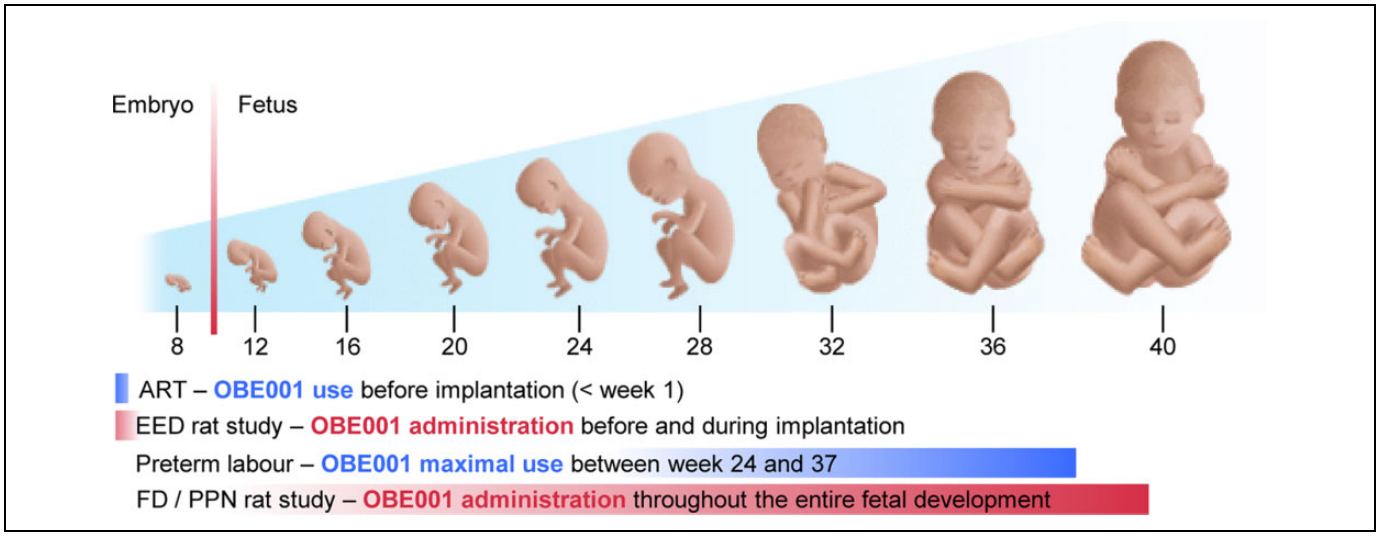

Figure 1. A comparison of the intended clinical use of OBE001 and the exposure scenarios used in the rat reproductive safety assessments relative to the state of human pregnancy. Pregnancy is split into embryonic (up to gestational week 10) and fetal development (beyond gestational week 10). Blue bars refer to the clinical use of OBE001, and red bars indicate the exposure scenarios used in rats. In the EED study, OBE001 was administered during follicle maturation, ovum release, mating, and implantation. ART indicates assisted reproductive technology; EED, early embryonic development; FD, fetal development; PPN, pre/postnatal development. (The color version of this figure is available in the online version at http://rs.sagepub.com/)

controls (15.77, 14.64, 14.36, 14.23 in the controls and at 37.5, 75 and 125 mg/kg/d, respectively). This finding was considered to be coincidental, as the number of implantations was well established prior to the start of dosing and the number of late resorptions and fetal deaths was minimal. Slightly higher mean placental weight did not achieve statistical significance and was considered to be associated with the lower mean number of fetuses per female and a single female with 1 fetus and a large placenta in the 125 mg/kg/d group. This finding was therefore considered to be unrelated to OBE001 administration. As for the fetuses, there was slightly but statistically significantly lower mean fetal weight at 75 and 125 mg/kg/d (3.71 and 3.65 g, respectively, vs 3.91 g in controls). This change was considered to be treatment related. No effect on mean fetal weight (3.88 g) was observed at 37.5 mg/kg/d.

There were no malformations or skeletal variations related to OBE001 administration, but a statistically significant increase in the percentage of fetuses with skeletal anomalies at 75 and 125 mg/kg/d (54% and 50%, respectively, vs 25% for controls) was observed. Relevant findings were related to incomplete ossification of 3 parts of the skull, the interparietal, parietals, and supraoccipital (Table 3). This finding was also present spontaneously in the control litters.

Overall, no effects on pup survival were observed for OBE001, and since there were no other observations of toxicological concern, the no observable adverse effect level for maternal toxicity, embryo FD, and pup survival and development to weaning for OBE001 were considered to be 125 mg/kg/d, and the NOEL was assigned as 37.5 mg/kg/d. Plasma exposures at study dose levels are presented in Table 2.

Discussion

The OTRan OBE001 is intended to improve embryo implantation and pregnancy rate in ART and to treat preterm labor. Our rodent reproductive safety testing strategy considered this anticipated drug use, and the presented studies were tailored to mimic the periods of exposure to the drug in clinical settings.

In the FER/EED study which emulated the exposure conditions of OBE001 in the ART indication, there were no relevant OBE001 effects. The NOEL was assigned as 125 mg/kg/d with exposures significantly exceeding those anticipated to be observed in ART patients. Clinical signs such as mouth rubbing, paddling, and salivation could be related to the dosing vehicle that contained the nonionic surfactant Labrasol (25%). As Labrasol is unlikely to have any endocrine activity, recorded clinical observations were considered to be associated with the taste of the Labrasol formulation as the catheter passed through the oral cavity. These clinical signs were consistent with the findings of the combined FD/PPN study in rats.

In the combined FD/PPN study, a small number of deaths across all groups including controls were seen. These were associated with pathology findings of respiratory tract edema and inflammation. In addition, a few dams in all groups showed dyspnea. It is likely that these findings can be related to the presence of Labrasol (50%) in the dose formulation as some data have indicated that inadvertent exposure, by aspiration into the lungs, could be irritating to epithelial cells. Instillation (aspiration dosing) of 0.02% or 1% Labrasol to the respiratory tract epithelium of mice for 1, 5, or 28 days with assessment of body weight and bronchoalveolar lavage fluid showed low pulmonary cytotoxicity and inflammation, which were more marked at 1%.[17] Systemically, however, Labrasol has low oral toxicity in rats with an median lethal dose (LD_{50}) value of 22 g/kg and is generally well tolerated following repeated dosing but has shown several occurrences of increased salivation and/or ploughing (excessive burrowing of the muzzle in the bedding material) for animals receiving 5% (0.53 g/kg/d) over 1 month.[17-19] The use of the Labrasol formulation is limited to preclinical studies, and OBE001 is administered as an oral dispersible tablet in a clinical setting. It was shown that OBE001 was rapidly absorbed using this formulation with pharmacokinetic properties suitable for treating women with threatened preterm labor.[12] For tocolysis, the oral dispersible tablet is currently considered to be the most suitable, safe, and patient-friendly drug preparation. Other drug preparations such as an intravenous dosage form to provide tocolysis in highly urgent cases or other drug preparations adapted for a more local (vaginal/cervical) application may be developed to address the needs of specific patient populations.

Administration of OBE001 from days 15 to 20 of gestation was characterized by a slight effect on mean body weight and food consumption for females at 125 mg/kg/d. In this group, there were a marginally increased number of females with slight body weight loss after 1 day of dosing, and this was reflected in mean body weight loss for the smaller FD subset. Since there was evidence of a recovery in mean body weight and food consumption by day 20 of gestation, this finding was considered not to be adverse. For females at 75 mg/kg/d, there was evidence of slightly lower mean food consumption after the start of dosing and a marginal effect on body weight gain between days 16 and 18 of gestation. These findings were transient and not considered adverse. There was no obvious effect on body weight gain for females at 37.5 mg/kg/d. Slight effects on food consumption for some animals after the start of dosing were considered not to be adverse.

In agreement with the reduction in mean fetal weight recorded for fetuses in the 75 and 125 mg/kg/d groups, retardation of some ossification parameters was seen at an increased incidence than the concurrent controls. It would appear, therefore, that the total number of fetuses with noteworthy retardation of skull bone ossification parameters was higher than might be expected from concurrent control data. However, the individual findings are of a type that occurred spontaneously in the control fetuses in this study. There was already partial ossification of the bone indicating that the anlage of the skull bones was present, and the ossification process was occurring and was not prevented by a developmental change in the fetuses. Ossification variations are commonly seen in fetuses that undergo cesarean section on day 20 of gestation as occurred in this study.[20] Indeed, it has been reported that the number of centers of ossification in rat fetuses is very limited in day 19 fetuses and varies considerably in day 20 fetuses but is homogeneous and uniform in day 21 fetuses.[21] In addition, the PPN subset pups that littered after at least a further 24 hours of gestation were found to have a similar body weight to controls. Mean pup body weight remained similar to the controls until weaning. If ossification of the skull bones had been prevented or delayed by administration of OBE001, it might be expected that there would be an effect on pup survival during the first few days postpartum. This was not seen from examination of the mean survival indices on day 4 of lactation and at weaning. Overall, the minor effect on fetal body weight can therefore be considered coincidental and the associated retardation of some ossification parameters not adverse.

It should be noted that although in a different situation, dosing of rats in a recent combined FD/PPN reproduction toxicology study (with sectioning on day 20 of gestation and littering and observation of offspring until day 25 of age) using a potential therapeutic vaccine (including a immunostimulant adjuvant) for cancer treatment showed an increase in incomplete ossification/unossified of cranial centers from use of the adjuvant ($n = 36$) among the sectioned fetuses compared to the saline control group ($n = 25$).[22] The study authors had no specific concern with this finding and reported that there was no indication of reproductive toxicity in this study.

The findings of our combined FD/PPN study in rats were comparable to the observations during a cross-fostering study in rats using the peptide OTRan atosiban.[23] Similar to OBE001, maternal treatment with atosiban had no effect on the number of offspring born, live birth index, or birth weight. However different to OBE001, atosiban results in an apparent increase

in the incidence of stillbirths and also an apparent decrease in subsequent neonatal survival.[24] McAnulty and Burns concluded that the poor viability observed in an earlier perinatal and postnatal study was attributable to maternal effects of atosiban, such as a failure of milk let-down, and not to neonatal toxicity.[23]

The risk of preterm birth resulting from ART is markedly higher than that of natural conceived pregnancies, and thus, these patients may need to receive OBE001 at the beginning and toward the end of pregnancy.[25] No additional translational rat studies will be necessary to cover this population, since the risk for the embryo or fetus results from the presence of the drug during an embryonic or fetal developmental stage rather than from the duration of treatment. However, should the indication of OBE001 be extended to preventive treatment in women with a history of preterm birth to reduce the risk of future preterm labor, additional studies may be necessary.

Overall, this work showed that OBE001 at up to 125 mg/kg/d had no effects on EED and no adverse effects on fetal and postnatal development of rats. Furthermore, plasma exposure at 125 mg/kg/d greatly exceeded the anticipated OBE001 exposure in women with preterm labor. These results constitute an important step toward the development of OBE001 in preterm labor and ART indications.

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: OP and AC are salaried employees of ObsEva SA. The FD/PPN study was performed at RBM, a company of the Merck Group, and LC was the study director of this study. The conduct and reporting of the FER/EED study and a review of the FD/PPN study data were contracted to Covance Laboratories Ltd. JR and DP were the study directors of this work, PB acted as a consultant to ObsEva. JR, DP, and PB are salaried employees of Covance Laboratories Ltd.

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Supplemental Material

The online [appendices/data supplements/etc] are available at http://rs.sagepub.com/supplemental

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