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Etravirine has no effect on fetal development in rats and rabbits

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Abstract

Background
The effect of etravirine (ETR; TMC125) on embryo-fetal and pre and postnatal development was assessed in pregnant rats and rabbits.

Methods
In the embryo-fetal study, ETR was administered at several oral doses up to 1000mg/kg/day in rats and 375mg/kg/day in rabbits during the period of fetal organogenesis (Gestation Day [GD] 6 to Day 16 in rats and Day 19 in rabbits). The uterus was examined and the number of implantations, live fetuses, fetal weight and sex distribution were analyzed. Fetuses were subject to external and visceral examination. In the pre and postnatal study, ETR was administered (up to 500mg/kg/day) to pregnant rats from Day 7 of gestation through Day 21 of lactation. The clinical conditions, sensory functions, and behavior and reproductive performance of the first generation were assessed.

Results
ETR showed no adverse effect on embryo-fetal development at any doses tested in rats or rabbits. ETR also had no effect on offspring development and maturation during lactation or postweaning in rats. In these studies, maternal systemic exposure to ETR was equivalent to the clinical exposure (area under the plasma concentration-time curve from time of administration to 24 hours after dosing; $AUC_{0\text{-}24h}$=7.4µg•h/mL) at the recommended therapeutic dose (200mg twice daily) at the end of treatment and was 2–3-fold higher at the beginning of dosing during the early stages of organogenesis.

Conclusions
These studies confirm that ETR was safe in pregnant rats and rabbits.

Background and aims

- ETR is a next-generation non-nucleoside reverse transcriptase inhibitor (NNRTI) with high potency against wild-type and drug-resistant HIV
- Three studies were conducted
  - two studies assessed embryo-fetal development in rats and rabbits
  - one study assessed pre and postnatal development in rats
- In the present analyses, the ETR preparations used were
  - the hydrogen bromide salt (ETR.HBr)
  - the commercial formulation (F060)*

*ETR base is amorphized by spray-drying technology using hydroxypropyl-methylcellulose as stabilizing polymer and microcellulose as inert carrier

Effect of ETR on embryo-fetal development in rats: study design

- Four groups of pregnant Wistar rats* received ETR.HBr‡ or control (vehicle), at a dose volume of 5mL/kg, once daily by oral gavage, during GD 6–16
  - control group: 0mg/kg/day (n=24)
  - ETR treatment groups
    - 250mg/kg/day (n=20)
    - 500mg/kg/day (n=22)
    - 1000mg/kg/day (n=20)
- 2–6 female satellite animals were included in each group for toxicokinetic analyses

*Wistar rats:Crl:BR (outbred, SPF-Quality) from Charles River (Germany)
‡ETR.HBr formulated in PEG 400

Effect of ETR on embryo-fetal development in rats: study design (cont'd)

- Regular observations were made for clinical signs, body weight and food consumption
- Dams were sacrificed on GD 21
- Macroscopic examination at necropsy was carried out on
  - sacrificed dams
  - all animals found dead prior to GD 21
- The uterus, cervix and ovaries were excised, weighed and examined for
  - gravid uterine weight
  - number of corpora lutea
  - implantations
  - live fetuses
  - fetal body weight
  - fetal sex distribution
- Evaluation of fetuses
  - all live fetuses were examined externally
  - alternate fetuses were preserved, eviscerated and stained with Alizarin red S for skeletal examination; remaining live fetuses were preserved in Bouin's fluid for visceral examination using a modified Wilson technique

ETR was not associated with any adverse effects on dams or fetal development in rats

- No mortalities were associated with ETR use
- No relevant clinical signs, or effects on body weight and food consumption, or on gross pathology were observed
- ETR had no effect on
  - gravid uterine weight
  - number of corpora lutea
  - number of pre-implantation losses
  - number of post-implantation losses
  - number of live implantations
  - fetal body weight
  - sex ratio
  - fetal abnormalities
- The maternal and embryo-fetal NOAEL is considered to be 1000mg/kg/day, based on the absence of any effect

NOAEL = No Observed Adverse Effect Level

Toxicokinetic data in female rats*

- Toxicokinetic analysis indicated nonlinear pharmacokinetics

ETR dose (mg/kg/day)	Sampling day	C_{max} (µg/mL)	$AUC_{0\text{–}24h}$ (µg•h/mL)
250	Day 11 (GD 16)	0.51	3.26
500	Day 11 (GD 16)	0.58	7.94
1000	Day 11 (GD 16)	0.57	8.27

- Exposures in rats are equivalent to those in patients at the recommended therapeutic dose (human C_{max} 0.45µg/mL and $AUC_{0\text{–}24h}$ 7.4µg•h/mL at 200mg bid)

*C_{max} and $AUC_{0\text{–}24h}$ values of ETR after repeated oral administration, GD 6–16
C_{max} = maximum plasma concentration

Effect of ETR on embryo-fetal development in rabbits: study design

- Four groups of sexually mature, time-mated female NZW rabbits* received ETR‡ or control (vehicle), at a dose volume of 15mL/kg, once daily by oral gavage, during GD 6–19
  - control group: 0mg/kg/day (n=20)
  - ETR treatment groups (all n=20)
    - 125mg/kg/day
    - 250mg/kg/day
    - 375mg/kg/day

*NZW rabbits: rabbits of the Hsd1F: NZW from Harlan (UK)
‡Spray-dried ETR formulated in water; NZW = New Zealand white

Effect of ETR on embryo-fetal development in rabbits: study design (cont'd)

- Regular observations were made for clinical signs, body weight and food consumption
- Dams were sacrificed on GD 28, and macroscopic examination at necropsy was performed
- The uterus was examined and the following analyzed
  - gravid uterine weight
  - number of corpora lutea
  - implantations
  - live fetuses
  - fetal body weight
  - fetal sex distribution
- All live fetuses were examined externally and viscerally (including the brain) by fresh tissue examination
- Fetuses were then eviscerated, preserved, stained with Alizarin red S and examined for skeletal abnormalities

Skeletal examination of rabbit fetuses

ETR was not associated with any adverse effects on dams or fetal development in rabbits

- No mortalities were associated with ETR use
- No relevant clinical signs were observed in dams following ETR use
- ETR at 375mg/kg/day resulted in
  - a slight reduction in body weight gain over the entire treatment period (GD 6–19)
  - reduced food consumption until GD 8 (≤32%)

ETR was not associated with any adverse effects on dams or fetal development in rabbits (cont'd)

- ETR had no effect on
  - gross pathology
  - gravid uterine weight
  - pregnancy rate
  - number of corpora lutea
  - number of pre-implantation losses
  - number of post-implantation losses
  - number of live implantations
  - fetal body weight
  - sex ratio
  - fetal abnormalities
- Maternal NOAEL is considered to be 125mg/kg/day, based on the effect on body weight and food consumption at the higher dose levels
- Embryo-fetal NOAEL is considered to be 375mg/kg/day, based on the absence of any relevant findings in this study

Toxicokinetic data in female rabbits*

ETR dose (mg/kg/day)	Sampling day	C_{max} (µg/mL)	$AUC_{0\text{–}24h}$ (µg•h/mL)
125	Day 1 (GD 6)	0.51	7.58
	Day 13 (GD 18)	0.38	6.09
250	Day 1 (GD 6)	0.63	11.6
	Day 13 (GD 18)	0.41	5.27
375	Day 1 (GD 6)	0.70	12.3
	Day 13 (GD 18)	0.54	9.67

- Exposures in rabbits are equivalent to those in patients at the recommended therapeutic dose (human C_{max} 0.45µg/mL and $AUC_{0\text{–}24h}$ 7.4µg•h/mL at 200mg bid)

*C_{max} and $AUC_{0\text{–}24h}$ values of spray-dried ETR after repeated oral administration, GD 6–19

Effect of ETR on maternal and pre and postnatal development in rats: study design

- Four groups of time-mated female Sprague-Dawley rats* received ETR‡ or control (vehicle), at a dose volume of 20mL/kg, once daily by oral gavage, during GD 7 to Day 21 of lactation
  - control group: 0mg/kg/day (n=28)
  - ETR treatment groups (all n=28)
    - 125mg/kg/day
    - 250mg/kg/day
    - 500mg/kg/day
  - offspring (first generation pups) were allowed to mature untreated
- Six time-mated female satellite animals were included in each group for toxicokinetic analyses

*Sprague-Dawley:Crl:CD BR from Charles River (UK)
‡Spray-dried ETR formulated in water

Effect of ETR on maternal and pre and postnatal development in rats: study design (cont'd)

- Regular observations were made for maternal clinical signs, body weight and food consumption
- Dams were allowed to litter, and nesting and nursing behavior monitored
- Parturition, litter size and numbers of each sex were recorded
- Clinical observations in pups and pup body weight were recorded
- Developmental landmarks were recorded for offspring; the number with
  - ears open
  - eyes open
  - preputial separation
  - vagina open
- Sensory function/reflexes were recorded for offspring
  - static righting reflex
  - startle response
  - pupillary light reflex
  - tail flick test
- Offspring were further assessed for
  - reproductive performance
  - behavior (learning, memory and locomotor activity)

Effect of ETR on maternal and pre and postnatal development in rats: study design (cont'd)

- Dams were sacrificed at litter weaning stage
  - macroscopic evaluation was performed on all principal females, including counting the number of implantation scars in each uterine horn
  - a range of tissue and organ samples were preserved, but not examined microscopically
- Macroscopic evaluation of offspring was performed
  - a range of tissue and organ samples were preserved, but not examined microscopically

ETR use had no effect on maternal and pre and postnatal development in rats

- No mortalities were associated with ETR use in dams
- No relevant clinical signs or effects on body weight or food consumption of dams were observed following ETR use
- Maternal treatment with ETR had no effect on
  - gestation
  - parturition
  - number of pups born
  - sex ratio
  - pup survival
  - clinical condition
- Body weight was higher in pups from ETR-treated animals than in controls
  - no clear dose response was observed
- Clinical condition, sensory function/reflexes, behavior and reproductive performance of offspring were unaffected by maternal treatment with ETR

ETR use had no effect on maternal and pre and postnatal development in rats (cont'd)

- No evidence for an effect of ETR use on macroscopic findings in dams or offspring
- Maternal NOAEL is considered to be 500mg/kg/day, based on the absence of any effect in this study
  - no delayed effects of maternal treatment on offspring selected for development to adulthood (assessed by physical development, sensory functions, reflexes, behavior, sexual maturation or reproductive performance)
- The NOAEL for pup development following maternal treatment with ETR is considered to be 500mg/kg/day, based on the absence of any effect in this study

Toxicokinetic data in female rats*

ETR dose (mg/kg/day)	Sampling day	C_{max} (µg/mL)	AUC‡ (µg•h/mL)
125	Day 1 (GD 7)	1.03	6.15
	Day 11 (GD 17)	0.65	5.29
250	Day 1 (GD 7)	1.30	9.42
	Day 11 (GD 17)	0.85	7.60
500	Day 1 (GD 7)	1.66	12.10
	Day 11 (GD 17)	0.64	3.63

- Exposures in rats are equivalent to those in patients at the recommended therapeutic dose (human C_{max} 0.45µg/mL and $AUC_{0\text{–}24h}$ 7.4µg•h/mL at 200mg bid)

*The C_{max} and AUC values of spray-dried ETR after repeated oral administration, GD 7–17
‡$AUC_{0\text{–}\infty}$ after single dose (GD 7) or $AUC_{0\text{–}24h}$ after repeated dose

Conclusions

- ETR use was not associated with adverse effects on embryo-fetal development in:
  - pregnant Wistar rats at levels up to 1000mg/kg/day
  - pregnant NZW rabbits at levels up to 375mg/kg/day.
- In both species, maternal exposure at the end of treatment was equivalent to human exposure at the recommended therapeutic dose:
  - exposure levels in the rabbit, however, exceeded clinical exposure at the beginning of dosing (during organogenesis), but subsequently declined due to autoinduction.
- ETR had no effect on offspring development in rats, during lactation and postweaning at doses ≤500mg/kg/day (equivalent to clinical exposure levels).
- Clinical data in pregnant humans are limited, and ETR should not be used during pregnancy unless the potential benefit justifies the potential risk.

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This poster is available on-line at www.tibotec.com