Juvenile Animal Study in Rats With the Aromatase Inhibitor Letrozole Administered for 9 Weeks including Recovery and Reproductive Phases

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Abstract

The aromatase inhibitor, letrozole, produces adverse effects on growth, skeletal development, sexual maturation, behavior, and reproductive anatomical and functional parameters when administered to rats during postnatal maturation, primarily through anti-estrogenic properties. Letrozole was utilized as a positive control to support validation of a juvenile animal toxicology program. Sprague-Dawley rats from untreated dams were administered vehicle (0.5% methylcellulose) or 0.05, 0.3, or 2.0 mg letrozole/kg once daily via oral gavage from postnatal days (PND) 4 through 70. They were evaluated for alterations of general toxicology/core endpoints, developmental landmarks, clinical and anatomic pathology, reproduction, and neurological development. The most prominent treatment-related effects were attributed to letrozole pharmacology. Body weight and weight gain of letrozole-treated animals were affected in all dose groups, being significantly lower in males and higher in females than concurrent controls. Female sexual maturation and reproduction were also affected in all letrozole groups. Vaginal opening was delayed by 3-4 days, and irregular estrous cycles occurred in 30-100% of females. Following cohabitation on PND 90 with males from the same dose group, all control females mated and were confirmed pregnant, while in females administered letrozole, mating indices were 0%, 11%, and 0% at 0.05, 0.3, and 2.0 mg/kg/day, respectively. Mean uterus weights were decreased at all letrozole doses, consistent with the microscopic finding of mild or marked uterine atrophy. Reproductive endpoints were within normal limits for males. Results of this study demonstrated juvenile toxicity consistent with known effects of letrozole.

Methods

Treatment	Test Article		Number of Offspring					
			Main Phase		Recovery Phase		Reproductive Phase	
	Dosage[a] (mg/kg)	Concentration (mg/mL)	M	F	M	F	M	F
Control	0	0	10	10	10	10	10	10
Letrozole	0.05	0.01	10	10	10	10	10	10
Letrozole	0.3	0.06	10	10	10	10	10	10
Letrozole	2	0.4	10	10	10	10	10	10

[a]The test article (Letrozole) and control (0.5 % methylcellulose) administered once daily via oral from PND 4 through 70±1.
M = Male F = Female

Table 1. Study Design. Offspring evaluations included general toxicology/core endpoints, developmental landmarks, clinical and anatomic pathology, reproduction, and neurological development. Main and recovery phase offspring were euthanized on PND 71±1 or PND 126±1, respectively. Organ weights, macroscopic, and microscopic findings were evaluated. Male and female reproductive phase animals from the same treatment group were cohabitated (1:1) on ~ PND 90 and evaluated for structural and functional effects on reproductive performance.

Results

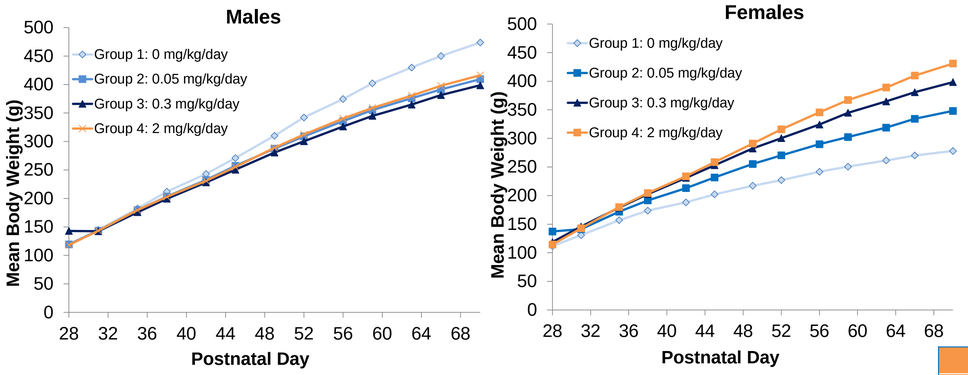

Figure 1. Body Weights: Body weights and weight gains were statistically significantly lower in males and higher in females of all dose Groups compared to controls.

Treatment Dosage (mg/kg)	Mean Day of Attainment		Estrus Cycling			
	Preputial Separation (Males)	Vaginal Opening (Females)	Cycling (%)	Regular[a] (%)	Number of Cycles	Cycle Length
Control (0)	46.00	31.48	100	90	3.2 ± 0.63	4.2 ± 0.63
Letrozole (0.05)	45.67	35.90	100	40	4.5 ± 1.72	4.66 ± 1.03
Letrozole (0.3)	45.41	35.79	100	66.7	4.33 ± 1.22	4.14 ± 0.43
Letrozole (2)	45.33	34.62	87.5	0	2.86 ± 1.57	4.23 ± 0.39

[a]Irregular estrus cycle = 5 diestrus, 3 estrus, 2 metestrus, or 2 proestrus in a row or a 2 day cycle

Table 2. Sexual Maturation: Sexual maturation was affected in females only. Vaginal opening was delayed in all letrozole treated groups relative to controls. Although not statistically significant, this was considered biologically relevant as body weights were increased for all treated females.
Estrous Cycling: The percentage of animals with regular estrous cycles was reduced in all letrozole-treated groups compared to controls. However, there was no overall effect on number of cycles or cycle length.

Treatment Dosage (mg/kg)	Mating Index (%)	Fertility Index (%)	Pregnancy Index (%)	Pre-coital Interval (Days)
Control (0)	100	100	100	3.0
Letrozole (0.05)	0	0	0	-
Letrozole (0.3)	11.1	11.1	11.1	-
Letrozole (2)	0	0	0	-

Table 3. Reproductive Performance: All letrozole-treated animals, with the exception of one female at 0.3 mg/kg/day, were non-gravid (compared to a 100% pregnancy rate for controls). The failure to breed was associated with treatment-related effects on the female reproductive tract that included delayed sexual maturation, disruption of the estrus cycle, and macroscopic and microscopic lesions of the ovaries and uterus.

Treatment Dosage (mg/kg)	Uterine Weight (% differences of group means from control mean)		
	Absolute	/Body	/Brain
Control (0)	-	-	-
Letrozole (0.05)	**-26**	**-39**	**-33**
Letrozole (0.3)	**-39**	**-58**	**-44**
Letrozole (2)	**-64**	**-77**	**-66**

Table 4. Uterine Weights: Mean uterine weights were statistically significantly decreased for females given ≥ 0.05 mg letrozole/kg/day, correlating with uterine atrophy.

	Group:	1	2	3	4
	Dose Letrozole (mg/kg/day):	0	0.05	0.3	2
Ovary	N:	10	10	9	10
Cyst, follicular, multiple, bilateral	mild	0	9	9	2
	moderate	0	0	0	8
Corpora lutea, decreased, bilateral	mild	0	1	1	0
	moderate	0	2	2	0
	marked	0	0	5	10
Uterus	N:	10	10	9	10
Atrophy, bilateral	mild	0	2	4	0
	marked	0	0	0	9
Mammary gland	N:	10	10	9	10
Hypertrophy/hyperplasia, epithelial cell, lobular	minimal	0	0	1	0
	mild	0	0	5	2
	moderate	0	0	2	3
Pituitary	N:	9	10	9	10
Hypertrophy, chromophobe, pars distalis, multifocal	minimal	0	3	3	2
	mild	0	1	6	6
	moderate	0	0	0	2
Vacuolation, chromophobe, pars distalis, multifocal	minimal	0	0	5	7
	mild	0	0	0	2

Table 5. Microscopic Findings: Histopathological findings are summarized. Letrozole-related microscopic findings were observed in the ovaries, uterus, and pituitary gland of females given ≥ 0.05 mg/kg/day, and in the mammary gland of females given ≥ 0.3 mg/kg/day.

Conclusion

Results of this study demonstrated juvenile toxicity consistent with the known pharmacologic effects of letrozole.

- Body weight and weight gain of letrozole-treated animals were affected in all dose groups; significantly lower in males and higher in females than controls.
- Sexual maturation and reproduction were affected in all letrozole-treated groups.
  - Vaginal opening was delayed by 3-4 days and irregular estrous cycles occurred.
  - Following cohabitation on PND 90 with males from the same dose group, all letrozole-treated females, with the exception of one female given the mid dose of 0.3 mg/kg/day, were non-gravid.
  - Mean uterine weights were decreased at all letrozole doses, consistent with the microscopic finding of mild or marked uterine atrophy.

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