FUNDAMENTAL AND APPLIED TOXICOLOGY 4, 977–982 (1984)

Teratogenic Dose–Response Relationships of Etretinate in the Golden Hamster[1]

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Teratogenic Dose–Response Relationships of Etretinate in the Golden Hamster. WILLIAMS, K. J., FERM, V. H., AND WILLHITE, C. C. (1984). *Fundam. Appl. Toxicol.* **4**, 977–982. Etretinate (Ro 10-9359; Tigason; 4-methoxy-2,3,6-trimethylphenyl analog of retinoic acid, ethyl ester) was evaluated for teratogenic activity in the Syrian golden hamster. Groups of pregnant hamsters were given a single oral dose of 2.8–88 mg/kg etretinate during the early primitive streak stage of gestation. No signs of maternal intoxication were observed in any of the hamsters given the retinoid and maternal body weight changes throughout gestation were not significantly different from those of the vehicle-treated group. Etretinate administration was associated with a dose-dependent increase in the incidence and severity of malformations. The average fetal body weight was significantly less in litters recovered from dams given 44 or 88 mg/kg of etretinate when compared to the average body weight of fetuses recovered from dams given an equivalent volume of the vehicle. The average crown–rump lengths also were significantly shorter in fetuses taken from the dams given 44 or 88 mg/kg etretinate as compared to the control group. The malformations induced by etretinate administration were similar to those noted following an oral dose of all-*trans*-retinoic acid (Willhite and Shealy, 1984). A comparison of the dose–response curves for induction of terata following treatment with etretinate or all-*trans*-retinoic acid revealed that etretinate was twice as potent as a teratogen in the hamster as all-*trans*-retinoic acid. Teratogenic activity of etretinate in the hamster was achieved at doses (mg/kg body wt) used in patients at current clinical therapeutic levels.

Vitamin A and its synthetic analogs (retinoids) have been investigated for their chemopreventive and possible chemotherapeutic effects in the treatment of cancer. There is extensive literature concerning the physiological and pharmacological effects of various retinoids in cell culture, organ culture, animal experiments, and clinical trials. However, efforts to determine the exact mechanisms of the retinoids in regulation of cellular growth and differentiation have not yet been successful (see Lotan (1980) for review).

Pharmacological dosages of naturally occurring forms of vitamin A have limited therapeutic value due to the accompanying hypervitaminosis A intoxication syndrome. This phenomenon has resulted in a search for synthetic retinoids with increased intrinsic biological activity and reduced toxicity (Sporn *et al.*, 1976, 1979; Bollag and Matter, 1981; Moon and McCormick, 1982). Etretinate (Fig. 1) is a synthetic analog in which the trimethylcyclohexenyl ring of the naturally occurring forms of vitamin A has been replaced by a substituted aromatic ring. Etretinate has been shown to have a high therapeutic ratio in comparison with all-*trans*-retinoic acid in cancer chemoprevention experiments (Bollag, 1974). Etretinate has also been reported to be well tolerated and

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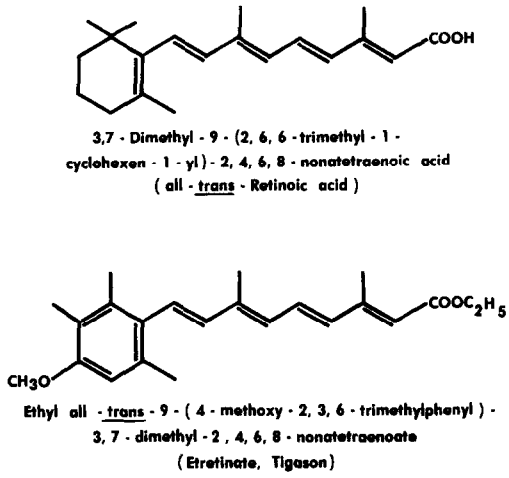

FIG. 1. Chemical structure of all-*trans*-retinoic acid and the aromatic analog etretinate.

thus clinically more effective than all-*trans*-retinoic acid in trials against actinic keratoses (Moriarty *et al.*, 1982), psoriasis (Goerz and Orfanos, 1978; Windhorst and Nigra, 1982), leukoplakia (Koch, 1978), T-cell lymphoma (Claudy *et al.*, 1982), and pulmonary metaplasia (Gouveia *et al.*, 1982).

The teratogenic effects of hypervitaminosis A in laboratory animals have been documented and numerous studies have described the malformations induced in the hamster by administration of naturally occurring forms of vitamin A (Marin-Padilla and Ferm, 1965; Marin-Padilla, 1966; Shenefelt, 1972; Marin-Padilla and Marin-Padilla, 1981). The present study was undertaken to evaluate the teratogenic potential of etretinate in the hamster and to compare the dose–response relationships of etretinate with those of all-*trans*-retinoic acid.

MATERIALS AND METHODS

Pregnant golden hamsters [Lak: LVG(SYR)] of known gestational age were purchased from the Charles River Breeding Laboratories (Wilmington, Mass.). The day following the evening of breeding was considered to be Day 1 of gestation. The hamsters were individually housed in polycarbonate cages with pine shavings for bedding. The animals were allowed tap water and laboratory chow (Ralston Purina Co., St. Louis, Mo.) *ad libitum*.

At 10:00 AM on the eighth day of gestation, the dams were given a single gastric intubation dose of 2.8–88 mg/kg etretinate (Ro 10-9359 (Tigason), Lot 002068; Hoffmann–LaRoche, Inc., Nutley, N.J.). The etretinate was prepared as a solution immediately before dosage by dissolving the retinoid in a small amount of reagent-grade acetone and diluting with polyoxyethylenesorbitan monolaurate (Tween 20, Lot 12F-0299, Sigma Chemical Co.) such that the final acetone concentration was no greater than 5%. The final dose volume was no greater than 0.5 ml/100 g body wt. An identical group of pregnant hamsters was given an oral dose of an equivalent volume of the vehicle. The etretinate was stored at −16°C in the dark under argon and handled under red light.

The hamsters were killed on Day 13 of gestation by CO_2 anoxia. The pregnant uteri were removed and the numbers of fetuses and resorbed conception sites were recorded for each dam. All fetuses were examined under a binocular dissecting microscope for gross external malformations (Ferm, 1967). Rib anomalies were noted by examination through the translucent skin prior to fixation (Ferm *et al.*, 1977). The crown–rump lengths of the fetuses were measured and the fetuses were dried on absorbent paper and weighed.

The litter was considered to be the experimental unit for statistical treatment of the data. The fetal body weight and crown–rump length data were analyzed using the one-way analysis of variance test and the probability calculated using the Newman–Keuls test (Snedecor and Cochran, 1967). Affected litters were considered to be those containing at least one malformed fetus. The statistical significance of the differences between doses was determined by χ^2 analysis with the Yates correction for continuity (Snedecor and Cochran, 1967), comparing each dose to the vehicle control. The maternal weight change was calculated from the day of treatment to the day of termination and those data were analyzed using the one-way analysis of variance test and the probability was calculated using the Newman–Keuls test (Snedecor and Cochran, 1967). The number of resorptions for each group was compared to the control using the Mann–Whitney *U* test (Snedecor and Cochran, 1967). Values were considered to be significantly different at the 0.05 probability level. The ED50 value for induction of terata was calculated using a computerized program (Spratt, 1966).

RESULTS

Oral administration of etretinate was associated with a significant increase in the number of litters containing abnormal offspring (Table 1). At a dose of 5.5 mg/kg or greater of etretinate, all of the litters contained at least one malformed fetus. There were

TABLE 1

THE EFFECT OF ETRETINATE ON HAMSTER EMBRYONIC DEVELOPMENT

Dose (mg/kg)	Dams treated	Affected litters[a] No.	Affected litters[a] %	Implantation sites	Resorptions No.	Resorptions %	Average fetal body weight (g ± SD)	Average crown–rump length (mm ± SD)	Average maternal weight change (g ± SD)
0	6	0	0	75	4	5.3	0.47 ± 0.10	15.6 ± 0.7	20.3 ± 4.2
2.8	8	4*	50	105	2	1.9	0.49 ± 0.06	16.0 ± 0.7	24.7 ± 3.5
5.5	8	8*	100	121	2	1.7	0.45 ± 0.07	15.3 ± 0.8	24.8 ± 3.9
11	8	8*	100	105	7	6.7	0.41 ± 0.05	15.0 ± 0.6	21.2 ± 4.0
22	8	8*	100	107	4	3.7	0.42 ± 0.05	15.1 ± 0.8	24.9 ± 7.6
44	9	9*	100	136	29	21	0.33 ± 0.05*	14.4 ± 0.6*	22.8 ± 4.3
88	4	2[b]	100	55	51*	93	0.27 ± 0.01*	13.3 ± 0.4*	21.5 ± 2.4

[a] Affected litters were considered to be those containing one or more malformed fetuses.

[b] Two litters contained malformed offspring. The two remaining litters were completely resorbed.

* Indicates $p < 0.05$ compared to control.

significant decreases ($p < 0.05$) in the average fetal body weight and the average crown–rump length following a dose of 44 or 88 mg/kg of etretinate. Maternal weight change from the day of treatment to the day of termination for etretinate-treated groups was not significantly different from that of the control (Table 1). At no point in the experiment were signs of illness noted in any of the dams. All fetuses that were not resorbed were alive. There was a significant increase ($p < 0.05$) in the resorption rate following a dose of 88 mg/kg etretinate.

Table 2 summarizes the incidence and types of malformations observed following an oral dose of etretinate in the hamster. There was a dose-dependent increase in the incidence of abnormal fetuses and in the

TABLE 2

TERATOGENIC EFFECTS CAUSED BY ETRETINATE ADMINISTRATION IN THE HAMSTER[a]

Dose (mg/kg)	Live fetuses	Fetuses with one or more malformations No.	Fetuses with one or more malformations %	CNS[b]	Exophthalmos	Mandible defect[c]	Rib[d]	Umbilical hernia	Limb[e]	Tail[f]
0	71	0	0	0	0	0	0	0	0	0
2.8	103	14	13.6	2	11	0	2	0	0	0
5.5	119	80	67.2	10	79	3	4	1	0	0
11	98	77	78.6	58	74	52	4	2	0	31
22	103	91	88.4	79	89	63	8	4	1	52
44	107	107	100	154	106	105	11	29	23	98
88	4	4	100	5	4	5	0	4	98	4

[a] See Table 1 for statistical treatment of these data.

[b] CNS defects include exencephaly, encephalocele, and occult spina bifida; some fetuses showed multiple CNS defects.

[c] Mandible defects include hypoplastic mandible (agnathia, micrognathia) and cleft mandible.

[d] Rib defects include rib fusion and crooked ribs.

[e] Limb defects include forelimb and hindlimb shortening and oligodactyly.

[f] Tail defects include aplastic, hypoplastic, and crooked tail.

severity of the malformations. Exophthalmos was the most common defect at the lower doses and central nervous system malformations, including exencephaly and occult spina bifida, predominated at the higher dosages with several fetuses exhibiting multiple central nervous system defects. Malformations of the extremities occurred only at the three highest doses (22, 44, and 88 mg/kg) and the severity of the limb anomalies tended to increase with an increase in the dose of etretinate.

The ED50 for induction of terata by administration of etretinate in the hamster was 5.7 mg/kg (5.1–6.2 was the 95% confidence limit). The ED50 value for induction of terata following administration of all-*trans*-retinoic acid to an identical population of pregnant hamsters (Willhite and Shealy, 1984) was calculated to be 10.1 mg/kg (9.0–11.1). The dose–response curves for these two retinoids were significantly parallel ($p < 0.001$) by χ^2 analysis, with a slope of 3.5, and the potency ratio was calculated to be 1.8 (1.5–2.0).

DISCUSSION

Administration of etretinate, an aromatic analog of all-*trans*-retinoic acid, was associated with significant teratogenic activity for the developing hamster embryo. Kamm (1982) and Hummler and Schupbach (1981) reported that etretinate administration was teratogenic for the rabbit, mouse, and rat at doses greater than 1, 2, and 4 mg/kg/day, respectively. Malformations were seen in the present study with hamsters following administration of a single oral dose of as little as 2.8 mg/kg (Tables 1 and 2). Clinical trials involving etretinate administration commonly employed oral doses ranging from 0.35 to 5.0 mg/kg/day (Rustin *et al.*, 1983; Cupissol *et al.*, 1982; Claudy *et al.*, 1982).

Studies on the metabolism of etretinate in rodents and humans have demonstrated that the major metabolite of the retinoid was the corresponding acid (Ro 10-1670; Paravicini *et al.*, 1981). The similar metabolic fate of etretinate in rodents and humans and the fact that retinoids have marked teratogenic activity in a wide variety of laboratory species, including nonhuman primates (Fantel *et al.*, 1977; Hendrickx *et al.*, 1980), suggests that the use of etretinate in women of childbearing age should be approached with caution. Human birth defects have been reported after etretinate (Peck, 1981) and other retinoid (Rosa, 1983) treatment during the first trimester of pregnancy.

The metabolic and functional interrelations of naturally occurring retinoids have been reviewed (Lotan, 1980). A free carboxyl group at position C15 was required for binding to cellular retinoic acid-binding protein, a protein which mediates the cellular and subcellular uptake of retinoic acid (Sani and Hill, 1976, Sani and Banerjee, 1981). The binding protein has been detected in all fetal rat organs studied, but was only marginally detectable in normal adult tissues (Lotan, 1980; Chytil and Ong, 1978, 1983). The enhanced teratogenic activity of etretinate in the hamster embryo as compared to that of all-*trans*-retinoic acid may be related, in part, to the acid metabolite's interaction with cellular retinoic acid-binding protein. In addition, the increase in teratogenic activity of etretinate as compared to all-*trans*-retinoic acid may be associated with the prolonged elimination half-life of etretinate (Paravicini *et al.*, 1981). The fact that the quantal dose–response curves for induction of terata by all-*trans*-retinoic acid and etretinate were parallel suggests that the two retinoids share a common mechanism of embryotoxic action. Further studies on the structure–activity relationships of the retinoids are required prior to construction of predictive structure–activity relationships for the retinoids in developmental toxicology.

ACKNOWLEDGMENTS

The authors are indebted to Dr. W. E. Scott of Hoffmann–LaRoche, Inc., for the gift of the etretinate.

This study was supported in part by NIH Grants ES-07104 (K.J.W.) and OH-01434 (V.H.F.) and USDA CRIS Unit 5102-20840-010 (C.C.W.) Ms. Williams is a Predoctoral Fellow of the Albert J. Ryan Foundation, Cincinnati, Ohio.

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