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Effects of Retinoic Acid on the Incidence of Neural Tube Defects and Other Malformations in Mice with the Quinky Gene

F. M. Abou-Tarboush
Department of Zoology, College of Science, King Saud University
P.O. Box 2455, Riyadh 11451, Saudi Arabia

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Abstract. Matings between normal young adult a^e/a^e female mice and heterozygous Quinky BALB/c males were used to study reported teratogenic and/or preventive effects of retinoic acid (RA) utilizing a mutation that produces neural tube defects (NTDs). The prenatal administration of RA at 10 or 20 mg/kg body weight increased the incidence of exencephaly and other malformations in fetuses with the Quinky gene. Neither curative nor beneficial effects of RA were observed on tail abnormalities at any dose rate used on day 8 or day 9 of gestation. On the contrary, RA proved to have, in addition to its teratogenic effect, lethal effects on fetuses.

Introduction

The semidominant Quinky gene of the mouse arose as a spontaneous mutation in the Q inbred line [1] and has variable effects on the skeleton, pigment and behavior of its carriers [2]. The gene is lethal in the homozygous state and most of the homozygotes either die soon after implantation or sometime later in pregnancy [1]. The heterozygotes, on the other hand, are viable and fertile, but have a kinky tail, shaker or circulating behavior, as well as pigment restriction that results in white hind toes [1,2].

The teratogenic effects of retinoic acid (RA) in rats [3–6], hamster [7–11] and mice [12–18] are well documented. However, some investigators have reported that RA might have a teratogenic and/or protective effect in fetuses genetically predisposed to neural tube defects (NTD) when administered to pregnant mice in various doses at different times during gestation [19–22]. Hence, an attempt has been made in the present study to investigate the effects of RA on mouse fetuses carrying the so called Quinky gene (genetically predisposed for NTD), when given at two dose levels to pregnant mice on day 8 or day 9 of gestation. This period is selected as it is the prime time for the drug to have its effects on the fetal developing neural tube.

Materials and Methods

Normal young adult a^e/a^e female mice and heterozygous Quinky (Q/+) BALB/c males were used in the study. They were housed in plastic boxes in an environmentally controlled room with a light/dark cycle of 14/10 hrs. In each box 3–4 a^e/a^e females were caged together with a BALB/c heterozygous Quinky male.

The commencement of pregnancy was determined by the detection of vaginal plugs in mated females and the day the plug was detected was counted as day zero of gestation. These females were then injected ip with 10 or 20 mg/kg of RA in corn oil on day 8 or day 9 of pregnancy. Control mice were injected ip with corresponding volumes of the corn oil vehicle. On day 17 of gestation, the mice were killed and the number of live fetuses and resorptions was noted in each mouse. Each fetus was then examined microscopically both externally and internally for gross developmental abnormalities. Ten fetuses from each group were then cleared and stained according to a slight modification of the method of McLeod [23] for skeletal examination.

The results were statistically analyzed using a 2 × 2 contingency table (x^2) and the Student t-test [24,25].

Results

The effects of RA on the fetuses obtained from pregnant a^e/a^e inbred mice are shown in Tables 1-4. Comparisons between RA-treated and control groups on day 8 of pregnancy are given in Table 1. The results of these comparisons shown that there was a significant increase in the proportion of resorption in the RA-treated groups at 20 mg/kg but no such effect was noticed at 10 mg/kg. The results of these comparisons also showed that there was a significant decrease in the mean litter size at the two dose levels used but no such effect in the mean body and placental weights.

Comparisons between RA-treated and control groups on day 9 of pregnancy are given in Table 2. The results of these comparisons showed that there were no significant differences betwen the RA-treated and the control groups in the proportion of resorption, mean litter size or mean body and placental weights for the two doses used.

Comparisons between RA-treated and control groups on day 8 or day 9 of pregnancy are shown in Table 3. The resutls of these comparisons show no significant differences between the RA-treated and control groups in the percent of Quinky fetuses for the two doses and the two days of treatment used.

Table 1. Effects of Treatment with 10 or 20 mg/kg retinoic acid on day 8 of gestation on fetal parameters.

Dose (mg/kg)	Control	10	20
No. of litters	6	8	8
No. of implantation	53	63	61
No. of live fetuses	51	58	23
No. of resorptions (%)	2 (3.77)	5 (7.94)	38 (62.30)**
Mean litter size ± SE (live)	8.50 ± 0.34	7.25 ± 0.71*	2.88 ± 052*
Mean body Wt. ± SE (g)	0.77 ± 0.015	0.81 ± 0.018	0.78 ± 0.020
Mean placental Wt. ± SE (g)	0.181 ± 0.018	0.168 ± 0.010	0.166 ± 0.007
Abns. observed†	None	None	3 ex.; 1 um. her.; 2 abn. hind. 2 ske. abn.

* Asterisks indicate values significantly different (* $P < 0.005$; ** $P < 0.001$) from the corresponding control.

† ex. = exencephaly, um. her. = umbilical hernia; abn. hind. = abnormal hindlimb; ske. abn. = skeletal abnormality.

Table 2. Effects of Treatment with 10 or 20 mg/kg retinoic acid on day 9 of gestation on fetal parameters.

Dose (mg/kg)	Control	10	20
No. of litters	6	8	8
No. of implantation	50	69	60
No. of live fetuses	49	64	54
No. of resorptions (%)	1 (2.00)	5 (7.25)	6 (10.00)
Mean litter size ± SE (live)	8.17 ± 0.70	8.00 ± 0.84	6.75 ± 0.62
Mean body Wt. ± SE (g)	0.74 ± 0.041	0.75 ± 0.016	0.81 ± 0.026
Mean placental Wt. ± SE (g)	0.164 ± 0.023	0.151 ± 0.012	0.149 ± 0.006
Abns. observed†	None	1um. her.; 1abn. hind. 1 ske. abn. 1mic.	1 tailless fetus; 2 ex.; 2 ske. abn.

* ex. = exencephaly; um. her. = umbilical hernia; abn. hind. = abnormal hindlimb; she. abn. = skeletal abnormality.

Table 3. Effects of treatment with 10 or 20 mg/kg retinoic acid on day 8 or day 9 of gestation on number and genotype of fetuses.

Dose (mg/kg)	Day of treatment	No. of litters	No. of live fetuses	No. of +/+ fetuses	No. of Q/+ fetuses (%)
Control	–	12	100	48	52 (52.00)
10	D8	8	58	31	27 (46.55)
20	D8	8	23	14	9 (39.13)
10	D9	8	64	31	33 (51.56)
20	D9	8	54	28	26 (48.15)

D8 = Day 8 of gestation; D9 = Day 9 of gestation.

Comparisons between the control groups and RA-treated groups on day 8 and 9 of pregnancy are given in Table 4. The results of these comparisons showed that there was a significant difference in the proportion of resorption and mean litter size between day 8 and day 9 at the dose level of 20 mg/kg of RA.

The results of this study, however, indicate that RA does induce some abnormalities (Tables 1 and 2) such as umbilical hernia, exencephaly, micrognathia and hindlimb and skeletal abnormalities, especially in some fetuses obtained from females treated with the 20 mg/kg dose level. However, no protective effects of the drug were noticed.

Table 4. Effects of treatment with 10 or 20 mg/kg retinoic acid on day 8 and day 9 of gestation of fetal parameters.

Dose (mg/kg)	Control		10		20	
Day of treatment	D8	D9	D8	D9	D8	D9
Mean litter size ± SE	8.5 ± 0.34	8.17 ± 0.70	7.25 ± 0.71	8.00 ± 0.84	2.88 ± 0.52	6.75 ± 0.62*
Resorportion (%)	2 (3.77)	1 (2.00)	5 (7.94)	5 (7.25)	38 (62.30)	6 (10.00)*

* $P < 0.001$.

Discussion

The present study has clearly demonstrated the deleterious and non-preventive effects of retinoic acid on developing mouse embryos whose mothers were treated with the drug during gestation. These dams were not obviously affected by the drug at any time of gestation. As in the observations of [12, 14–18], RA triggered the production of neural tube defects (NTDs) in the form of exencephaly and tail abnormalities, especially when administered at the dose of 20 mg/kg either on day 8 or day 9 of gestation. Moreover, and in line with the observations of [13, 15, 17], a significant increase in fetal mortality, as measured by resorption per implantation site and a significant decrease in the mean litter size, was observed in the RA-treated groups. This lethal effect was especially conspicuous when the drug was adminsitered at the rate of 20 mg/kg on day 8 of gestation. However, a lower mortality rate occurred when the drug was administered on day 9 of gestation. This might indicate that some crucial and essential events might have taken place in the process of development on day 8. Moreover, it is known fact that most fetuses have elevated sensitivity to teratogens during organogenesis, making embryos highly vulnerable to death, but such sensitivity decreases as development proceeds [21] and this might be accounted for the different results noticed in the percent of resorption and the decrease in mean litter size between day 8 and day 9, especially at the dose level of 20 mg/kg of RA.

Abnormalities similar to those observed in the present study, as well as other types of malformations, have been previously observed with RA [12-14, 16-18].

However, contrary to the observations of [19-22], no preventive effects on tail abnormalities produced by the Quinky gene have been noticed at any dose rate nor at any day of drug administration in the present study. The discrepancy between the present resutls and those of [19-22] could well be due to the nature of the mutant used. This is because many genes affect the mouse tail differently [26]. Hence, not all NTDs spontaneously induced by various mutatins could be corrected or prevented by an exogenous agent such as RA. Similar observations have recently been made [27]. The preventive effects of the agent might be dependent on the nature or causation of the mutant investigated and on the time on which the defects are first initiated.

The primary effects of RA are not known, but it has been observed to have many secondary effects such as promotion of cellular differentiation, stimulation of epithelial hyperplasia, alteration of cell-cycle time and glycolipid synthesis, as well as the modification of cell-membrane integrity [20]. Such secondary effects might be responsible for the deleterious effects observed in the present study. Moreover, recent studies also indicated that RA can modify the pattern of cell differentiation in the central nervous system [28, 29].

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تأثيرات فيتامين أ (حمض الريتنوك) على مدى حدوث تشوهات الأنبوب العصبي الوراثية والتشوهات الخلقية الأخرى في الفئران حاملة الجين المسبب لالتواء الذيل

فيصل محمد أبو طربوش

قسم علم الحيوان، كلية العلوم، جامعة الملك سعود، ص.ب ٢٤٥٥،
الرياض ١١٤٥١، المملكة العربية السعودية

(استلم في ٢٠/١/١٤١٢هـ؛ قبل للنشر في ٢٢/٨/١٤١٢هـ)

ملخص البحث. استعملت في هذه الدراسة تزاوجات بين إناث يافعة عادية (a°/a°) وذكور خليطة للجين المسبب لالتواء الذيل المسمى بـ Quinky gene من سلالة BALB/c لدراسة التأثيرات الضارة و/أو العلاجية لحمض الريتنوك وذلك باستخدام طفرة تنتج عيوب الأنبوب العصبي في فئران المعامل. ولقد أوضحت الدراسة أن حقن هذه المادة للأمهات خلال فترة الحمل وخاصة بالجرعات ١٠ أو ٢٠ مغم/كغم من وزن الجسم قد أدى إلى زيادة تشوهات الأنبوب العصبي الوراثية (وخاصة المرتبطة بمنطقة الرأس) والعيوب الأخرى في الأجنّة المتحصل عليها من هذه الأمهات بالمقارنة بالمجموعة الضابطة. كما أوضحت هذه الدراسة بأنه ليس لهذه المادة أية تأثيرات علاجية على تشوهات الأنبوب العصبي الوراثية بالجرعات المستخدمة خلال اليوم الثامن أو التاسع للحمل، بل على العكس فقد اتضح أن لهذه المادة تأثيرات ضارة وتأثيراً مميتاً على الأجنّة المتحصل عليها.