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Influence of gestational age at exposure on the prenatal effects of gamma-radiation

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The objective of this investigation was to evaluate the influence of gestational age at exposure on the prenatal effects of gamma-radiation. Pregnant ICR mice were exposed to a single dose of 2.0 Gy gamma-radiation at a gestational 2.5 to 15.5 days post-coitus (p.c.). The animals were sacrificed on day 18 of gestation and the fetuses were examined for mortality, growth retardation, change in head size and any other morphological abnormalities. The only demonstrable effect of irradiation during the pre-implantation period was an increase in prenatal mortality. Resorptions were maximal on post-exposure day 2.5 after conception. The pre-implantation irradiated embryos which survived did not show any major fetal abnormalities. Small head, growth retardation, cleft palate, dilatation of the cerebral ventricle, dilatation of the renal pelvis and abnormalities of the extremities and tail were prominent after exposure during the organogenesis period, especially on day 11.5 of gestation. Our results indicate that the late period of organogenesis in the mouse is a particularly sensitive phase in terms of the development of the brain, skull and extremities.

Key words: radiation, malformation, mouse, gestational age

Introduction

Irradiation of mammalian embryos can produce a spectrum of morphological changes, ranging from temporary stunting of growth to severe organ defects and death [2]. During the period of major organogenesis, mammalian embryos are highly susceptible to radiation-induced gross anatomic abnormalities. In the mouse this period is from 7 to 12 days p.c., corresponding to about 14 to 50 days in humans [5]. The abnormalities induced depend on the organs undergoing differentiation at the time of the irradiation, the stage of differentiation and the radiation dose [1].

The effect of irradiation during the early period of murine development, one-cell to the blastocyst stage, has been extensively studied *in vitro* by Streffer and co-workers [17-19, 24, 25] and *in vivo* by Russell, Rugh and others [6, 10, 26, 27, 31, 32]. The induction of malformations by exposure during major organogenesis and the early fetal periods have received considerable attention in early radiation embryology [7, 8, 21, 23, 31, 39] and continues to be a subject of interest [11, 14, 33, 34]. In a review, Mole argued that the concept of critical periods based on marked responses to high doses may not be applicable to lower doses [16]. Despite numerous published studies on radiation teratology [2, 36], relatively little information is available on the relationship between radiation dose and the incidence of specific abnormalities. Therefore, we undertook to systematically study periods of high sensitivity and the dose-incidence relationships of the prenatal effects of radiation.

Materials and Methods

Animals

ICR mice were maintained under controlled temperature and light conditions, on standard mouse food and water *ad libitum*. Virgin females and males, 10-12 weeks of age, were randomly mated overnight. Females with a vaginal plug were separated in the morning and marked as 0 day pregnant. All the mice were killed on day 18 p.c. by cervical dislocation.

Irradiation

The pregnant mice were exposed to 2.0 Gy gamma-radiation at dose-rate of 10 Gy/min on any one gestation day from 2.5 to 15.5 days p.c.

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Table 1. Observations on the 18th day of mouse fetuses exposed to 2 Gy gamma-radiation on different gestation days.

Observations	Exposure day p.c.					
	Control	2.5	5.5	7.5	11.5	15.5
No. of mother	6	6	6	6	6	6
No. of implants	74	74	76	82	86	74
No. of embryonic death	3	1	6	15[a]	5	3
No. of fetal death	2	0	1	2	1	0
No. of resorption	0	48[d]	10[b]	20[d]	0	0
Prenatal mortality No. (%)	5(6.76)	49(66.22)[d]	17(22.37)[a]	37(45.12)[d]	6(6.98)	3(4.05)
Live fetuses	69	25	59	45	80	71
GRF No. (%)	5(7.25)	2(8)	41(69.49)[d]	30(66.67)[d]	80(100)[d]	22(30.99)[c]
Body weight (g)	1.59±0.09	1.61±0.12	1.33±0.06[d]	1.26±0.23[d]	0.92±0.08[d]	1.44±0.01[d]
Body length (cm)	3.45±0.63	3.60±0.93	3.23±0.42[a]	3.10±0.40[b]	2.71±0.22[d]	3.21±0.65[a]
Head length (cm)	1.15±0.05	1.16±0.02	1.12±0.01[d]	1.07±0.04[d]	1.02±0.04[d]	1.17±0.02[a]
Head width (cm)	0.84±0.02	0.84±0.02	0.79±0.01[d]	0.79±0.07[d]	0.72±0.02[d]	0.81±0.01[d]
Incidence of decreased head length	2.90	4.0	3.39	44.44	72.5	1.41
Incidence of decreased head width	2.03	8	49.15	53.33	98.75	28.17

GRF: Growth retarded fetuses, calculated as the number of growth retarded fetuses/total number of live fetuses. Fetuses weighing less than two standard deviations of mean body weight of the control group were considered as growth retarded.
A head width or length of less than two standard deviations of mean control value was defined decreased head width or length.
[a-d]Difference from the control. [a]$p<0.05$, [b]$p<0.005$, [c]$p<0.001$, [d]$p<0.0001$.

Prenatal mortality

Uterine horns were opened and observed for the total number of implantations including resorption, embryonic death and fetal death. (A) Resorptions: included (a) implantation failure, where the implantation site was marked by a rudimentary fleshy mass, not a full placentum, and (b) cases where only a placentum was present, with no attached embryonic rudiments. (B) Embryonic death: partly formed embryo found attached to placental disc. (C) Fetal death: fully formed dead fetuses, distinguished by a darker colour, and macerated fetuses which were pale in color and soft to the touch. Pre-implantation loss, if any, with no identifying mark on the uterine wall, was not estimated in this study.

Fetal anomalies

Live fetuses were removed from the uterus, cleaned and observed for any externally detectable developmental anomalies. Fetuses were weighed individually and the mean fetal weight of the individual group litter was calculated. Fetuses weighing less than two standard deviations of the mean control group body weight were considered as growth-retarded. Body length was measured from the tip of the snout to the base of the tail. The longitudinal distance from the tip of the snout to the base of the skull was recorded as head length. The distance between the two ears was recorded as head width. Measurements were made with a vernier callipers. All fetuses were checked for external malformations under dissection microscope. Fetuses were fixed in Bouin's solution, then stored in 70% ethanol. The presence of visceral malformations was determined using Wilson's cross-sectional technique [38]. Alizarin red-S and alcian blue staining were used to examine skeletal malformations [9].

Results

A significant increase in prenatal mortality was observed when the irradiation was performed on pre-implantation days 2.5 p.c. and 5.5 p.c., maximum effect was observed on day 2.5 p.c. The early organogenesis stage (day 7.5 p.c.) was also highly sensitive. Exposure at the late organogenesis and fetal stage did not result in any significant increase in mortality (Table 1).

Exposure on days 5.5, 7.5 or 11.5 p.c. produced significant increases in the number of growth retarded fetuses. A non-significant increase was observed after exposure during the pre-implantation (day 2.5 p.c.) period. A significant decrease in the mean fetal weight was observed after exposure during the stages of organogenesis (days 7.5 and 11.5 p.c.), but this effect was not pronounced after exposure on the fetal period, day 15.5 p.c. (Table 1). Although the embryos appear to be sensitive to this effect throughout the period of preimplantation and organogenesis (days 5.5-11.5 p.c.), the lowest head size was recorded when exposure occurred on gestation day 11.5 (Table 1).

Malformations are summarized in Table 2. From the data presented in Table 2, it can be seen that a malformed fetus usually had more than one anormaly. The commonest types of malformations were a cleft palate (Fig. 1), dilata-

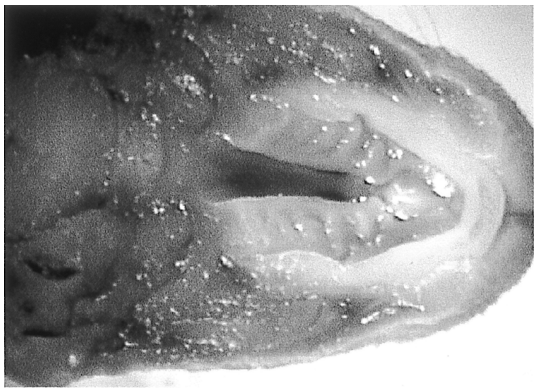

Fig. 1. Malformed palate of mouse fetus: cleft palate.

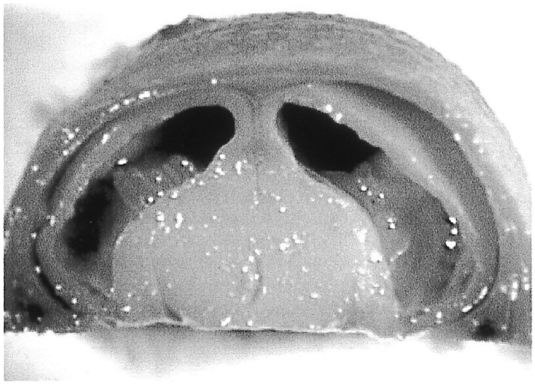

Fig. 2. Malformed head section of mouse fetus: dilation of lateral ventricles.

Fig. 3. Malformed kidney: dilation of renal pelvis.

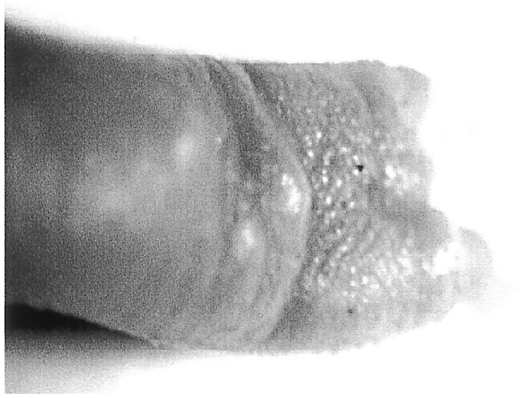

Fig. 4. Malformed digits of mouse fetus: ectrodactyly.

tion of the cerebral ventricle (Fig. 2), and dilatation of the renal pelvis (Fig. 3), moreover, abnormalities of the extremities (Fig. 4) and tail were prominent after exposure during the organogenesis period, especially on gestational day 11.5. Other anomalities were observed in any of the exposed groups, but the number of cases was too small to show the nature of any causal relationships.

Discussion

The present work is a systematic study of the comparative radiosensitivity of different gestational ages to acute irradiation, as assessed by detectable effects in full-grown mouse fetuses.

Our finding that pre-implantation exposure results in resorptions, while those embryos which survive this effect develop into normal fetuses without any apparent damage, agree with the conclusions of Russell [30, 31] and Uma Devi and Baskar [33]. Maximum lethality was found after exposure on day 2.5 p.c. A similar observation was made by Rugh and Wohlfromm [28], using x-rays. Based on the stage classification of mouse development in relation to the day p.c. [22], the present results on pre-implantation exposure indicate that the morula stages (day 2.5 p.c.) have highest sensitivity to the lethal effect of radiation. This sensitivity decreased after day 5.5 p.c. Muller et al. [20] also failed to observe any significant increase in prenatal death after 1 Gy exposure on day 4 p.c. The sensitivity to radiation killing decreased as the blastocyst progressed, but again there was a period of high sensitivity during the organogenesis period, day 7.5. Irradiation at this stage resulted in a significant increase in prenatal mortality, mainly due to resorption and embryonic death. A highly sensitive phase for embryonic lethality during the early organogenesis has been reported for mice after acute exposure to 2 Gy X-rays [11]. The significant increase in total mortality, observed in our study, after exposure on day 7.5 p.c. had a larger component of embryonic death than caused by exposure at the earlier stages. Sensitivity to the lethal effects of radiation decreased during the fetal period, as was also reported by Konermann [11] and Rugh and Wohlfromm [28], and supports the earlier conclusions of Russell [29] and others [25, 33, 37] that the period of organogenesis is less sensitive to the lethal effects of radiation.

The number of growth-retarded fetuses was higher after

Table 2. Malformations in 18-day fetuses exposed to 2Gy gamma-radiation on different gestation days.

	Exposure day p.c.					
	Control	2.5	5.5	7.5	11.5	15.5
External malformation						
Fetus examined	69	25	59	45	80	71
Ablepharon	0	0	0	1(2.22)	0	0
Micrognathia	0	0	0	1(2.22)	0	0
Gastroschisis	0	0	0	1(2.22)	0	0
Omphalocele	0	0	0	2(4.44)	0	0
Kinky tail	0	0	1(1.69)	2(4.44)	14(17.5)	0
Branchyury	0	0	0	1(2.22)	3(3.75)	1(1.41)
Rudimentry tail	0	0	0	1(2.22)	0	0
Digits	0	0	0	12(26.67)	72(90)	1(1.41)
Anal atresia	0	0	0	0	1(1.25)	0
Internal malformation						
Fetuses examined	35	13	31	22	41	37
Dilatation of cerebral ventricle	0	0	2(6.45)	9(40.91)	26(63.41)	0
Stenosis of nasal cavity	0	0	0	1(4.55)	1(2.44)	0
Cleft palate	0	0	0	0	15(36.59)	0
Dextrocardia	0	0	0	3(13.64)	0	0
Levorotation of heart	0	0	1(3.23)	4(18.18)	2(4.83)	0
Abnormal lobation of lung	0	0	0	3(13.64)	1(2.44)	0
Detect of diaphragm	0	0	0	1(4.55)	0	0
Diaphragmatic hernia	0	0	0	3(13.64)	0	0
Dilatation of renal pelvis	0	2(15.38)	10(32.26)	7(31.82)	3(7.32)	0
Skeletal malformation						
Fetuses examined	34	12	28	23	39	34
Deformity of occipital bone	0	0	1(3.57)	2(8.70)	1(2.56)	0
Splitting of cervical vertebrae	0	0	0	4(17.39)	0	0
Abnormal arrangement of cervical vertebrae	0	0	0	1(4.35)	0	0
Abnormal ossification of coccygeal vertebrae	0	0	0	0	1(2.56)	0
Fusion of lumbar vertebrae	0	0	0	0	1(2.56)	0
Abnormal arrangement of lumbar vertebrae	0	0	0	1(4.35)	0	0
Fusion of thoracic vertebrae	0	0	0	0	2(5.13)	0
Absence of ribs	0	0	0	2(8.70)	0	0
Fusion of ribs	0	0	0	4(17.39)	0	0
Bifurcation of ribs	0	0	0	2(8.70)	0	0
Shortening of ribs	0	0	0	2(8.70)	0	0
Displasia of sternebrae	0	0	0	0	1(2.56)	0
Missing of sternebrae	0	0	0	0	1(2.56)	0
Hypoplasia of sternebrae	0	0	0	3(13.04)	3(7.69)	0
Curvature of tibia	0	0	0	0	1(2.56)	0
Absence of metatarsal bone	0	0	0	0	5(12.82)	0
Absence of metacarpal bone	0	0	0	0	15(38.46)	0
Absence of clavicle	0	0	0	1(4.35)	0	0
Malformed offspring	0	2(8)	14(23.73)[a]	35(77.78)[a]	78(97.5)[a]	2(2.82)

[a]Difference from the control at $p<0.0001$.

gamma-exposure during the entire organogenesis period, but maximal retarded fetuses were produced by irradiation on day 11.5 p.c. A significant reduction in mean fetal weight was also seen in fetuses exposed during the later period of organogenesis, days 7.5-11.5 p.c., which agrees with the findings of Konermann [11] that the greatest loss in weight was caused by irradiation on day 10 or 11 p.c., and conforms with the data from Russell [31] and Kriegel et al. [12]. Exposure during the fetal stage of day 15.5 p.c. also resulted in significantly lower fetal weight, indicating that susceptible fetuses at this stage are as vulnerable to the stunting effect of radiation as at the later organogenesis period, but a comparatively lower number are affected. Small head size has been reported to be a prominent effect in the Japanese children exposed between 4 and 17 weeks of gestation [15]. A significant decrease in head size was also observed after irradiation at day 11.5 p.c. both with x-rays and gamma-rays in mice [34, 35]. In the present study a noticeable decrease in head size (both length and width) was also evident after exposure between days 5.5 and 15.5 p.c., but the maximal head shorten was seen after exposure on day 11.5 p.c. Head width was also similarly reduced after exposure at this stage.

The most common types of malformations resulting from gamma-irradiation were cleft palate, dilatation of the cerebral ventricle, dilatation of the renal pelvis and abnormalities of the extremities and tail, which were prominent after exposure during the organogenesis period, especially on day 11.5 of gestation. The abnormalities of the extremities were brachydactyly, ectrodactyly, polydactyly, and syndactyly, which would not have been severe defects in postnatal mice [13]. From the data presented in table 2, it can be seen that a malformed fetus usually has more than one anormaly. Some mice had many abnormalities on the same forepaw(s) and/or hindpaw(s). Individual fetuses with many abnormalities on the foreleg and /or hindleg were counted as one. Abnormalities of the extremities were more frequent than cleft palate after irradiation. These results are in agreement with earlier studies [3, 4, 13] that maximal abnormality frequency is found after exposure during the organogenesis period. Other anomalities were observed in any of the exposed groups, but the number of these cases was too small to indicate a causal relationship.

Our results indicate that the late period of organogenesis in the mouse is a particularly sensitive phase in the development of brain, skull and extremities.

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