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Dose-Incidence Relationships on the Prenatal Effects of Gamma-Radiation in Mice

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ABSTRACT

The objective of this investigation was to evaluate dose-incidence relationships on the prenatal effects of gamma-radiation. Pregnant ICR mice were exposed on day 11.5 after conception, coincident with the most sensitive stage for the induction of major congenital malformations, with 0.5-4.0 Gy of gamma- radiations. 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. With increasing radiation dose, incidence of small head, growth retarded fetuses, cleft palate, dilatation of cerebral ventricle and abnormalities of the extremities in live fetuses rose. The threshold doses of radiation that induced cleft palate and dilatation of cerebral ventricle, and abnormal extremities were between 1.0 and 2.0 Gy, and between 0.5 and 1.0 Gy, respectively.

Key words : Radiation, Malformation, Dose-incidence relationship, ICR mouse

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; this period spans 7 to 12 days post-coitus (p.c.) in mice, corresponding to about 14 to 50 days in humans [5]. The induced abnormalities 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. This led us to carry out a systematic study on the highly sensitive prenatal periods and the dose-incidence related to 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 a single whole-body gamma-irradiation with 0.5, 1.0, 2.0 and 4.0 Gy at dose-rate of 10 Gy/min on day 11.5 after conception. Gamma rays were delivered from a Co-60 source (Gamma-Cell 3000 Elan, Nordion International, Canada).

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

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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 color, 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

An increase in mortality was seen in the study on dose-incidence response, but the increase was significant only after exposure to 4.0 Gy. (Table 1). An increase in the number of growth retarded offspring was seen at 0.5 Gy which increases further with radiation dose. A similar effect was seen in the growth parameters, with significant decrease in mean body weight, body length and head size (Table 1).

Malformations were summarized in Table 2. From the data presented in Table 2, it shows that a malformed fetus usually had more than one anormaly. The most common types of malformations resulting from gamma-irradiation were cleft palate, dilatation of cerebral ventricle, dilatation of renal pelvis and abnormalities of the extremities and tail.

With increasing radiation dose, cleft palate, dilatation of cerebral ventricle and abnormalities of the extremities in live fetuses rose (Table 2). Other anomalities were observed in any of the exposed groups.

Table 1. Observation on the mouse fetuses 18 days after exposure to different doses of gamma-ray on 11.5 day of gestation.

Observations	Dose (Gy)				
	0	0.5	1.0	2.0	4.0
No. of mother	6	7	7	6	6
No. of implants	74	109	116	86	93
No. of embryonic death	3	3	0	5	3
No. of fetal death	2	1	0	1	6
No. of resorption	0	5	6	0	24[b]
Prenatal mortality					
No. (%)	5(6.76)	9(8.26)	6(5.17)	6(6.98)	33(35.48)[b]
Live fetuses	69	100	110	80	60
GRF					
No. (%)	5(7.25)	41(41)[b]	67(60.91)[b]	80(100)[b]	60(100)[b]
Body weight (g)	1.59±0.09	1.41±0.12[b]	1.34±0.15[b]	0.92±0.08[b]	0.62±0.11[b]
Body length (cm)	3.45±0.63	3.53±0.13	3.41±0.16	2.71±0.22[b]	2.50±0.24[b]
Head length (cm)	1.15±0.05	1.13±0.05[a]	1.09±0.05[b]	1.02±0.04[b]	0.89±0.06[b]
Head width (cm)	0.84±0.02	0.80±0.05[b]	0.76±0.04[b]	0.72±0.02[b]	0.61±0.03[b]
Incidence of decreased head length	2.90	48	79.09	72.5	100
Incidence of decreased head width	2.03	3	26.36	98.75	100

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 as decreased head width or length.

[a], [b] Difference from the control. [a]$p<0.005$, [b]$p<0.0001$.

Table 2. Malformations in 18-day fetuses exposed to different dose of gamma-ray on 11.5 days of gestation

	Dose (Gy)				
	Control	0.5	1	2	4
External malformation					
Fetus examined	69	100	110	80	60
Omphalocele	0	1(1)	0	0	0
Kinky tail	0	0	0	14(17.5)	2(3.33)
Brachyury	0	0	1(0.91)	3(3.75)	58(96.67)
Club foot	0	0	0	0	12(20)
Digits	0	0	12(10.91)	72(90)	60(100)
Dwarf	0	0	0	0	60(100)
Anal atresia	0	0	0	1(1.25)	0
Hematoma	0	4(4)	6(5.45)	0	11(18.33)
Internal malformation					
Fetuses examined	35	52	58	41	32
Dilatation of cerebral ventricle	0	0	0	26(63.41)	32(100)
Stenosis of nasal cavity	0	0	0	1(2.44)	0
Cleft palate	0	0	1(1.72)	15(36.59)	27(84.38
Levorotation of heart	0	0	0	2(4.83)	0
Abnormal lobation of lung	0	0	0	1(2.44)	0
Dilatation of renal pelvis	0	2(3.85)	0	3(7.32)	4(12.5)
Skeletal malformation					
Fetuses examined	34	48	52	39	28
Fusion of cervical vertebrae	0	0	0	0	1(3.53)
Deformity of occipital bone	0	0	0	1(2.56)	4(14.29)
Splitting of cervical vertebrae	0	1(2.08)	1(1.92)	0	0
Separating of cervical vertebrae	0	2(4.17)	0	0	0
Abnormal ossification of coccygeal vertebrae	0	0	0	1(2.56)	0
Fusion of lumbar vertebrae	0	0	0	1(2.56)	0
Fusion of thoracic vertebrae	0	0	0	2(5.13)	1(3.57)
Absence of ribs	0	0	1(1.92)	0	7(25)
Fusion of ribs	0	0	0	0	6(21.43)
Wavy ribs	0	1(2.08)	4(7.69)	0	8(28.57)
Hypoplasia of ribs	0	0	0	0	27(96.43)
Displasia of sternebrae	0	0	0	1(2.56)	0
Missing of sternebrae	0	0	0	1(2.56)	0
Hypoplasia of sternebrae	0	0	0	3(7.69)	0
Curvature of tibia	0	0	0	1(2.56)	0
Absence of metatarsal bone	0	0	1(1.92)	5(12.82)	28(100)
Absence of metacarpal bone	0	0	0	15(37.46)	28(100)
Malformed offspring	0	9(9)[a]	22(20)[b]	78(97.5)[c]	60(100)[c]

[a-c] Difference from the control. [a]$p<0.05$, [b]$p<0.005$, [c]$p<0.0001$.

Discussion

The present work is the systematic study of dose-incidence relationships to acute irradiation, assessed by detectable effect in the full-grown mouse fetuses.

A higher than normal incidence of prenatal mortality was observed after exposure to 0.5 Gy, but the increase became statistically significant only after 4.0 Gy. This is in support of 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 and significant reduction in mean fetal weight were seen in fetus exposed on day 11.5 p.c. Our results are well correlated with the findings of Konermann [11] that the greatest loss in weight was caused by irradiation on day 10 or 11 p.c., which also conform to the data from Russell [31] and Kriegel et al. [12]. Small head size has been reported to be a prominent effect in the Japanese children exposed during the 4-17 weeks of gestation [15]. Significant decrease in head size was a feature 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. The shorting of head was seen after exposure on day 11.5 p.c. Head width was also similarly reduced after exposure at this stage. An increase in the number of growth-retarded offspring was seen at 0.5 Gy, which increases further as the radiation dose increased. A similar effect was seen in the growth parameters, including a significant decrease in mean body weight, body length and head size.

The most common types of malformations resulting from gamma-irradiation were cleft palate, dilatation of cerebral ventricle, dilatation of renal pelvis and abnormalities of the extremities and tail were prominent after exposure on day 11.5 of gestation. The abnormalities of extremities were brachydactyly, ectrodactyly, polydactyly, and syndactyly, which would not have been severe defects in postnatal mice [13]. In this study, with increasing radiation dose, cleft palate, dilatation of cerebral ventricle and abnormalities of the extremities in live fetuses rose. From the data presented in table 2, it can be seen that a malformed fetus usually had more than one anormaly. Some mice, especially those irradiated with high doses, had many abnormalities on the same forepaw(s) and/or hindpaw(s). The fetuses which had many abnormalities on the foreleg and /or hindleg were counted as one. The number of fetuses with abnormal extremities was significantly higher in the groups exposed to radiation at a dose 1.0 Gy or more. The abnormalities of the extremities were more frequent than cleft palate after irradiation. These results are in agreement with earlier studies [3, 4, 13] that the maximal frequency was found after exposure during organogenesis period, and at this period a dose-dependant increase was observed. Other anomalities were observed in any of the exposed groups. The number of these cases was too small to indicate a causal relationship with exposure.

The result indicated that the late period of organogenesis in the mouse is a particularly sensitive phase in the development of brain, skull and extremities. The threshold doses of radiation that induced cleft palate and dilatation of cerebral ventricle, and abnormal extremities were between 1.0 and 2.0 Gy, and between 0.5 and 1.0 Gy, respectively.

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