

Transgenerational Transmission of Radiation Damage: Genomic Instability and Congenital Malformation¹

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Genomic instability/Malformation/Transgenerational effects/Preconceptional irradiation/Prenatal irradiation.

The congenital malformation gastroschisis has a genetic disposition in the inbred mouse strain HLG/Zte. It is increased after preconceptional irradiation of males or females. Radiation exposures during the meiotic stages are most efficient. This malformation can also be induced by ionising radiation when the exposure takes place during the preimplantation period especially during the zygote stage. This latter effect can be transmitted to the next mouse generation. Other macroscopically visible or skeletal malformations are not significantly induced under these experimental conditions. These latter malformations are increased by radiation exposures during major organogenesis. The mechanisms for the development of the effects are different. Radiation exposure of the mouse zygote (1 to 3 hours p.c.) also leads to the induction of genomic instability in skin fibroblasts of the fetus. This phenomenon also occurs in a mouse strain (C57BL/6J) which is not susceptible to radiation-induced gastroschisis during the preimplantation period. The genomic instability is transmitted to the next mouse generation. During genomic instability chromatide breaks are dominating as in non-exposed cells. With respect to “spontaneous” malformations gastroschisis is dominating in HLG/Zte mice. Late radiation effects seem to have similar patterns as observed in non-exposed subjects, however, the rates are increased after irradiation.

INTRODUCTION

Genetic effects of ionising radiation have been of high interest with respect to the risk estimation over many decades since it has been observed by Muller that such effects could be induced in *Drosophila melanogaster*.¹⁾ Present risk estimates are based on experimental data mainly with mice.²⁾ Various biological endpoints of transgenerational effects have been analyzed in this connection and congenital malformations are one class of these endpoints.³⁾ Malformations can be mainly induced by ionising radiation in mammals when the exposure takes place during the major organogenesis and it has been postulated that exposures during other phases of prenatal development do not cause such malformations.⁴⁾ However, it has been found that in certain mouse strains malformations can also be induced by ionising radiations as well as by genotoxic substances when the exposures take place during the preimplantation period. In

our group it could be demonstrated that the mechanism of these processes is based on a genetic predisposition and that this radiation-induced damage can also be transmitted to the next generation of mice.

During these investigations it was also found that chromosomal aberrations are increased in fetal fibroblasts although the radiation exposure had taken place many cell generations earlier and this phenomenon was described as “instability of the genome”.⁵⁾ The induction of congenital malformation also occurred after preconceptional irradiation of males as well as of females. These data will be summarized in the following.

MATERIALS AND METHODS

Female and male mice of the strain HLG/Zte, the inbred version of the originally used “Heiligenberg-strain” (derived from NMRI mice) and was colony-bred, was used throughout most the experiments. In some experiments mice of the C57BL strain were used. The animals were kept on a standard diet in an alternating 12 h light-dark cycle. Fertilization occurred on the day of estrus 2 h after mating had started at 6 a.m. Females with a vaginal plug were segregated. In case of postconceptional irradiation radiation exposure took place 1 or 3 hour post conception (1 or 3 h p.c.).

Animals were exposed to X-rays (Stabilipan X-ray

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machine Siemens, Erlangen, Germany; 240 kV, 15 mA, 0.5 mm copper filter, dose rate 1 Gy/min) or to ^{137}Cs gamma-rays with a dose rate of 0.28 Gy/h.^{5,6)}

For the cytogenetic studies preimplantation embryos or skin biopsies were obtained from fetuses on day 19 p.c., metaphases were prepared and analysed under the microscope in the usual way.⁵⁾

RESULTS

Induction of congenital malformations

The inbred Heiligenberger mouse strain, which had been established in the "Radiologisches Institut" of Freiburg University, Germany during the 1940s and 1950s and further bred in a colony at the Institute of Medical Radiobiology at the University Clinics in Essen, Germany since 1975 (registered as HLG/Zte), has a frequency of congenital malformations with a rate of around 3 to 4 percent.⁷⁾ The dominating malformation is gastroschisis, a hernia of the bowel, with 1 to 2 percent in this mouse strain. Besides some skeletal malformations and gastroschisis only very few other macroscopically visible malformations are found.

It was observed that this type of malformation (gastroschisis) was specifically increased by exposure to X-rays and cyclotron neutrons (average energy 6 MeV). This effect occurred even when the radiation exposure took place during the preimplantation period, especially after exposure of the zygote.⁷⁻⁹⁾ Such an effect was unexpected and against the rules which have been postulated earlier.⁴⁾ However, in further investigations at the same and later times similar observations were reported with other mouse strains after exposure to ionising radiation or genotoxic chemicals.⁴⁾

In further investigations it was found that according to the former postulates no radiation-increased rate of congenital malformations occurred when C57BL/6J mice were irradiated at the zygote stage^{9,10)} and such an effect was also not seen after radiation exposure of zygotes which resulted from cross breeding of C57BL/6J and HLG/Zte mice. Thus no radiation-induced increase of gastroschisis was found in HLG \times C57BL mice after irradiation during the preimplantation period. These data gave first indications that the malformation gastroschisis has a genetic disposition in HLG/Zte mice and that it has apparently a recessive genetic trait. The analysis with backcross animals [(HLG \times C57BL) \times HLG] demonstrated that two to three genes are involved in the genetic process. The following linkage analysis revealed that at least one of the responsible genes is located on mouse chromosome 7 close to the marker D7Mit195, a region in which genes for imprinting are observed.¹¹⁾

Therefore it was not surprising that an increase of the rate of gastroschisis also occurred after preconceptual Gamma- or X-irradiation.^{6,12)} Mature female mice were irradiated with 1 to 3 Gy X-rays in these experiments and mated with non-irradiated male mice 1 to 4 weeks later (irradiation 1 to

4 weeks before conception). The number of gastroschisis increased with increasing radiation dose and reached statistical significance after a radiation dose of 2 Gy. The effect was highest when the mating took place 1 week after radiation exposure.¹²⁾ Under these conditions only gastroschisis and no other macroscopically visible congenital malformations were found. The survival of fetuses was lowest when irradiation occurred 4 weeks before ovulation.

The studies with preconceptual irradiation of male mice with 2.8 Gy gamma-rays yielded a decreased survival of fetuses when the irradiation took place 1 to 7 weeks before mating, no radiation effect was observed on fetal survival with an interval of 8 weeks. The lethal events were mainly preimplantation death and early resorptions. As with preconceptual irradiation of females congenital malformations also increased after preconceptual irradiation of males. In the first place the meiotic stages of spermatogenesis were affected by radiation exposure (Table 1). The effect was highest when the radiation exposure occurred 4 weeks before mating and no significant effect was observed with an interval of 8 weeks.

The data after preconceptual irradiation of males show even more stringent than those with females that these effects have a genetic basis as possible indirect effects connected to radiation sickness or other extragenetic effects after irradiation of females can be entirely excluded.⁶⁾ However, the irradiation of females apparently results in higher effects than the irradiation of males (Table 1). This is the case for the survival of fetuses as well as for the induction of gastroschisis. Again gastroschisis is the only observed macroscopically visible malformation.

As has already been pointed out gastroschisis could also significantly be induced by exposure of zygotes (1 to 3 hours p.c.) to X-rays or to cyclotron neutrons.⁷⁾ A significant effect occurred after 0.50 Gy neutrons or 1.0 Gy of X-rays. The analysis of the dose effect relationship gave a linear-quadratic dose dependence without a threshold for both radiation qualities. Other skeletal malformations were slightly but not significantly increased.⁷⁾ The first significant increases of lethal events were observed after radiation doses of 0.12 Gy for neutrons and of 0.25 Gy for X-rays. These events were mainly preimplantation deaths and early resorptions.

The irradiation of later stages of preimplantation (2-cell-stage to blastocyst) yielded a threshold dose for the induction of malformations (gastroschisis) and in general the radiation effect was smaller than during the first hours of zygote development (Table 1).^{8,9)} It is interesting that the radiosensitivity with respect to induction of malformations decreases during and briefly after implantation of the embryos and also the type of malformations changes. Thus the ratio of gastroschisis to exencephaly (malformation with the second highest rate in HLG mice) is 19.3 : 1 in unirradiated controls, 15.5 : 1 after irradiation during the preimplantation period and reaches 1.2 : 1 after implantation (day 8 p.c.).⁸⁾

Table 1. Surviving and malformed fetuses after exposure to ionizing radiation at various stages (Preconceptional, Zygote, Preimplantation Period [2-cell to blastocyst] and "Trans-generational") (HLG mice)

Stage of Exposure	Rad. D. (Gy)	Living Fetuses		
		N	%	Malf. Fet. %
Control	0	2.511	87	1.5
Preconc. (F) 1-4w	3.0	763	26	17.3**
Preconc. (M) 1w	2.8	103	39	5.5*
Preconc. (M) 4w	2.8	247	48	6.9**
Preconc. (M) 8w	2.8	249	77	2.4
Zygote	1.0	147	49	7.8**
PreImpl.-m	1.0	309	78	2.2
PreImpl.-m	3.0	457	34	28.8**
Transgenerat.	1.0	462	76	4.4

* Significantly different from controls at $P < 0.05$

** Significantly different from controls at $P < 0.01$

Abbrev.: Preconc. (F): Preconceptional irradiation of females 1 to 4 weeks before mating;
Preconc. (M) 1w/4w/8w: Ppreconceptional irradiation of males 1,4 or 8 weeks before conception;
PreImpl.-m: Preimplantation period (multicellular: 2-cell to blastocyst);
Transgenerat.: Irradiation of zygotes and investigation of next generation.

This finding strongly indicates the differences of the mechanisms for the formation of these malformations by radiation exposures during the preimplantation period or major organogenesis.

In further experiments mouse zygotes of the HLG/Zte strain were X-irradiated with 1 Gy. The healthy looking mice were grown-up to 8 to 12 weeks old mature mice. The females of these litters were mated with non-exposed males. The fetuses of this second generation after irradiation were isolated on day 19 p.c. by Caesarian section and the fetuses as well as the uterus content were analysed.¹³⁾ It was observed that significant increases occurred in early and late resorptions as well as for the number of sterile females in the second generation after radiation exposure.^{13,14)} The frequency of fetuses with a gastroschisis showed also an increased number, but this was just on the borderline below significance (Table 2).

However, it was interesting that in dams which had developed from radiation exposed zygotes frequently two fetuses with gastroschisis (9 out of 21 dams), developed, whereas this occurred very rarely in those dams which had not been irradiated in the zygote stage (1 out of 7 dams) (Table 3). Such an event of two malformed fetuses in the same litter also occurred less frequent in the non-exposed controls as well as in the 1st generation which had been irradiated in the stage of the zygote. Again gastroschisis was the only observed macroscopically visible congenital malformation

Table 2. Teratogenic effects in unirradiated controls and in litters of the second generation after X-irradiation of HLG zygotes 1 h postconception with 1000 mGy X-rays (14)

Type of effect	Irradiated group (%)	Controls (%)
Gastroschisis	6.5	3.5
Early resorptions	18.0*	9.8
Late resorptions	2.4*	0
Sterile individuals	62*	34

* Statistically significant with $P < 0.01$

Table 3. Distribution of malformations among mated females of the first and second generation (13) (HLG mice)

Mated females of	Females with at least one malformation	Females with		
		1 malform.	2 malform.	3 malform.
1st generation				
Control	18	17	1	
Exposed	46	42	3	1
2nd generation				
Control	7	6	1	
Exposed	21	12	9*	

* Significantly different at $P < 0.05$ when compared to the controls and at $P < 0.01$ when compared to the first generation

in these experiments.

Induction of genomic instability

The mammalian preimplantation embryo is a very instructive experimental object, as it can be very nicely observed under the microscope and decided how many cell divisions have taken place after the irradiation of the zygote (1-cell stage). Thus it was found that chromosomal aberrations can be seen not only in 1st mitosis after radiation exposures but also further new chromosomal aberrations are formed in the 2nd as well as in the 3rd mitosis after irradiation.^{15,16} In further studies fibroblast cultures were established from skin biopsies which were obtained from murine fetuses on day 19 p.c. Chromosomal aberrations were measured in these fibroblast cells 48 hours after the start of the *in vitro* culture.

When the fibroblasts were harvested from fetuses which had been X-irradiated in the zygote stage the rate of chromosomal aberrations was significantly higher than in the fibroblasts that were obtained from fetuses which had not obtained any radiation exposure at all. This effect was found not only in the HLG/Zte mice but also in the C57BL/6J mouse strain.¹⁴ It was clearly dependent on the radiation dose in the dose range of 0.5 to 2.0 Gy and the dose response was even stronger in the C57BL/6J mice in comparison to the HLG/Zte mice (Table 4). This phenomenon was reported for the first time in 1989 and was termed “genomic instability”.⁵ It was stated: “Apparently the radiation exposure of the zygote had led to an instability of the genome so that

‘spontaneous’ aberrations occur more frequently”.⁵)

It was very interesting further that in the first mitotic division after radiation exposure of the zygote (mitosis from the 1-cell to the 2-cell stage) chromosome breaks were dominating over chromatide breaks,¹⁴ whereas in the fetal fibroblasts with the radiation exposure many cell generations earlier the rate of chromatide breaks was higher than that of the chromosome breaks.^{5,14} Thus it can be concluded: While the pattern of chromosomal aberrations was shifted in the 1st mitosis after irradiation towards aberrations of the type of chromosome breaks, in mitoses with genomic instability the aberrations with chromatide breaks were dominating as it was also the case in mitoses with ‘spontaneous’ aberrations (Table 5).¹⁴)

This genomic instability measured on the chromosome level was also found in the next mouse generation which had not been exposed to ionising radiation. In these experiments the chromosomal aberrations were measured in the peripheral blood lymphocytes of mature female mice (10 weeks old) which had been exposed to 1 Gy X-rays at the zygote stage (1st generation). An increased genomic instability was observed in blood cells from mice which were exposed in the zygote stage in comparison to lymphocytes from non-exposed mice of the HLG/Zte strain. Further the chromosomal aberrations were measured in the fibroblasts from skin biopsies of fetuses (2nd generation) which were born from the mating of zygote-exposed females (1st generation) with non-exposed male mice. A significant increase of chromosomal aberrations developed in the fibroblasts from the

Table 4. Chromosome aberrations in fibroblasts of fetuses (19 days postconception) of C57BL/6J and HLG mice irradiated as zygotes 1 h postconception (14)

Mouse Strain	Control		500 mGy		1000 mGy		2000 mGy	
	Aberrat./ cells	%	Aberrat./ cells	%	Aberrat./ cells	%	Aberrat./ cells	%
C57BL/6J	22/795	2.8	81/648	12.5	136/626	21.7	109/400	27.5
HLG	29/400	7.3	66/674	9.8	48/400	12.0	56/322	17.4

Table 5. Frequency of chromosome- and chromatide-type aberrations in fetal mouse cells from zygotes irradiated 1 h postconception (HLG mice)

	Chromosome-type (aberrations/cell)	Chromatide-type (aberrations/cell)	Ratio (chromosome: chromatide)
First mitosis after exposure (1- to 2-cell embryo)			
Control	0.013	0.022	0.59
Irradiated	0.316	0.118	2.68
Fibroblasts (19 days postconception)			
Control	0.03	0.043	0.70
Irradiated	0.078	0.122	0.64

Table 6. Chromosomal aberrations (CA/100 metaphases) in lymphocytes of adult HLG mice (1st generation) and in skin fibroblasts of mouse fetuses (2nd generation) after X-irradiation of zygotes (1st generation) (3 h p.c.)

	CA/100 metaph. 1 st generation	CA/100 metaph. 2 nd generation
Control	1.6 ± 0.5	2.5 ± 0.8
X.irrad.	3.2 ± 0.8**	6.8 ± 1.5**

***P* < 0.01 (Controls against X-irradiated)

fetuses which originated from the females exposed in the zygote stage (Table 6).

DISCUSSION

The reported data show that a transgenerational transmission occurs for radiation-induced congenital malformations as well as for genomic instability, the latter measured on the chromosome level. Similar data have been reported earlier by Nomura³⁾ as well as by Kirk and Lyon.¹⁷⁾ With male mice the effect of preconceptional irradiation was most efficient when the radiation exposure took place at the meiotic stages (Table 1) which was also observed by Nomura.^{18,19)} It further seems to be of interest that in our studies a malformation is involved with a genetic background where possibly genes with imprinting activities are involved.

With respect to malformations such a transgenerational effect is only observed in our experimental system with that malformation which has the above mentioned genetic background. Only this and no other macroscopically visible malformation is induced by ionising radiation after a preconceptional exposure of male mice or female animals. In the same way this is also caused by radiation exposures during the preimplantation period especially during the zygote stage (Table 1). For the latter experimental conditions it was also studied whether skeletal malformations were increased and no significant effect was found in the dose ranges of 0.25 to 2.0 Gy X-rays or 0.12 to 0.75 Gy cyclotron neutrons.⁷⁾

Table 1 gives a summary of the extent to which congenital malformations (gastroschisis) are caused by ionising radiation at the various stages. It clearly shows that the effect per Gy is highest after the exposure during the preimplantation period and this especially during the zygote stage. These latter conditions are certainly of higher interest in the lower dose range as apparently a dose response curve without a threshold describes the data best. Therefore this design as well as the preconceptional irradiation are very appropriate condition in order to study experimental effects with a genetic basis and show a much more pronounced effect than experiments with another design.

Other malformations as exencephaly etc. which are observed after in utero irradiation during the major organo-

genesis are not induced under these conditions.⁴⁾ The mechanisms of the development of congenital malformations after preconceptional and preimplantation irradiation are apparently quite different from those which develop after irradiation during major organogenesis. The described shift of the ratio gastroschisis over exencephaly after irradiation during the preimplantation period or the major organogenesis is a strong indication for this statement.

Thus the pattern of types of "spontaneous" malformations is identical with the pattern of malformations which are observed after irradiation before conception and during the preimplantation period. This finding is in agreement with an observation for genetic mutations which was already made by Muller in 1927 who reported that only such mutations occurred in his famous experiments with *Drosophila* after irradiation which were also seen "spontaneously".¹⁾

In this respect it is further interesting that the pattern of increased chromosomal aberrations in fetal cells with genomic instability resemble that one which is observed without radiation exposure, whereas directly after irradiation a different pattern appears (Tab. 5). For the development of genomic instability cells apparently have to go through many cell cycles. This effect also occurs after preconceptional irradiation^{20,21)} although this experimental design was not used here. The mechanism of the development of genomic instability is not resolved up to now and several possibilities are discussed.^{14,20,21)}

From these data it may be assumed that the manifestation of radiation effects which are expressed at later times and not directly after radiation exposures develop from the involvement of "weak regions" in the mammalian genome. After the random absorption of the radiation energy and after the random distribution of primary damage in the genome besides DNA repair a processing of the non-repaired or mis-repaired damaged sites in the genome takes place over several cell cycles. This residual damage then possibly manifests into mutations, congenital malformations and cancer which can also develop from endogenous processes as well as from exposures to other exogenous factors. On this basis it appears explainable why radiation-induced late effects cannot be distinguished from those phenomena which are observed "spontaneously" in the studied biological systems including humans.

The development of genomic instability after radiation exposure is a very intriguing phenomenon and this effect may be involved in the mechanisms for the manifestation of radiation late effects. From the data presented here it must, however, be concluded that the development of genomic instability may contribute to and facilitate the expression of the congenital malformation but this effect is not sufficient for the development of such a radiation late effect. The specific genetic disposition of the HLG mice is necessary for the radiation-induced causation of gastroschisis. The development of genomic instability is apparently a much more universal phe-

nomenon. A genomic instability was also found in C57BL/6J mice but no gastroschisis was caused in this mouse strain after irradiation during the preimplantation period.

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