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RECIPROCAL TRANSLOCATIONS AND REPRODUCTIVE CAPACITY IN RABBITS FOLLOWING EXTERNAL GAMMA IRRADIATION

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Summary

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The induction of reciprocal translocations (RT) and the reproductive capacity in sexually mature male New Zealand White rabbits were followed out after external gamma irradiation within the dose range of 0.5 Gy – 2.5 Gy.

The analysis of results about the litter size in irradiated males, mated with non-irradiated females as a marker of their reproductive capacity, performed 30 days after the irradiation, showed a reduction with 20% and on the 40th day – by 46%. Spermatozoa, irradiated in post spermatogonial cell stages were tested within post irradiation days 10 and 40. The cytogenetical data of irradiated spermatogonia and the mutations at the stage spermatocyte in diakinesis metaphase I evidenced the presence of ring and chain quadrivalents, the incidence of RT being higher after irradiation at 2.5 Gy.

Key words: ionized radiation, radiation mutagenesis, reciprocal translocations, reproduction, spermatogenesis

INTRODUCTION

Ionized radiation, being one of the environmental cytotoxic factors, causes germinative cellular death and therefore, sterility (Oakberg, 1975; Meistrich, 1993b) from one side whereas from the other, induces genetic changes in the genetic material (Meistrich, 1993a; Byrne *et al.,* 1998; Ahmadi, 1999), manifested at different levels as genetic mutations, chromosomal aberrations, germ cells with unbalanced genome and consequent effects in the progeny as dominant lethal mutations (DLM), congenital malformations etc.

Male individuals, submitted to external gamma radiation with higher than the background to sublethal doses (depending on the rate of absorbed dose and the time post irradiation), undergo a period of sterility followed by remission, both processes being function of the rate of damage of the pool of stem cell in testes (Hasegawa *et al.*, 1997; De Rooij, 2001).

It is known that the sterile period is resulting from the radiation-induced cellular death of spermatogonia and spermatocytes (Searle, 1981), that, on their part, result in

reduction in the next germ cell generations.

Despite the numerous studies in this field, the concentration of spermatozoa in semen when the fertilization of ova decreases or stops as well as the degree of spermatogenesis impairment when some of ovulating ova could be fertilized and others – not fertilized, are unknown.

The elucidation of the dose-time-effect phenomenon is especially important with regard to the evaluation of the risk for the next generations due to the mutagenic effect of ionized radiation.

The decrease of the average number of liveborn offspring of one female is used as an indicator of the appearance of DLM (Russel *et al.*, 1998), caused by disturbances with postimplantation and/or preimplantation components. The first are due to the increased incidence of dead zygotes and the latter are indicated by the differences in the number of yellow bodies in the ovary and the number of implanted zygotes.

The aim of the present study was to determine the incidence of reciprocal translocations (RT) induced in spermatogonia and the size of litters produced from spermatozoa irradiated in various stages of the spermatogenesis in rabbits exposed to various doses of gamma radiation.

MATERIALS AND METHODS

The experiment was performed with 78 sexually mature New Zealand White rabbits from both genders at the age of 4.5-5.0 months. All animals were of equal body weight (4.0-4.5 kg) and were fed with complete granulated diet for breeding rabbits and housed in individual cages prior to and during the experiment.

Male rabbits (n=18) were assigned to the following experimental groups (6 animals each): group I – rabbits irradiated with 0.5 Gy, group II – rabbits irradiated with 2.5 Gy and group III – non-irradiated (control) rabbits – sham-irradiated.

The animals from groups I and II were subjected to whole-body irradiation using a ^{60}Co gamma equipment (Rokus) at dose density of 24 cGy/min.

Three months after the irradiation, the testes of irradiated rabbits were used for making chromosomal preparations for detection of RT, induced in spermatogonia. They were determined in the spermatocyte stage in diakinesis metaphase I according to the method of Evans *et al.* (1964), modified for rabbits by the Laboratory of Radiation Genetics at the National Centre of Radiobiology and Radioprotection.

Female intact rabbits (n=60) were divided into 4 experimental and one control groups (12 animals each).

The experimental does were mated with male rabbits irradiated at 2.5 Gy at different intervals post treatment in order to obtain offsprings from spermatozoa following irradiation at different stages of spermatogenesis as followed: groups IV, V, VI and VII – female rabbits mated with irradiated males at post irradiation days 10, 20, 30 and 40, respectively; group VIII – female rabbits mated with non-irradiated (control) males.

The average number of liveborn rabbits from one doe in the experimental groups vs that in controls was used as a parameter of the reproductive capacity of males.

A cytogenetical examination of rabbit testes was performed. A total number of 600 metaphase plates from 3 intact rabbits and 1200 metaphase plates from 6 rabbits

irradiated at 0.5 and 2.5 Gy were examined.

The statistical analysis of data was performed by the t-test for independent samples (STATISTICA).

RESULTS AND DISCUSSION

Reciprocal translocations

The cytogenetical examination of testes of non-irradiated rabbits showed absence of RT in their germ cells. The respective data, obtained in male rabbits irradiated within the dose range 0.5–2.5 Gy are presented in Table 1.

The analysis of results in 0.5 Gy rabbits showed the presence of multivalents as ring quadrivalents (Fig. 1), and their percentage was 0.83±0.16. The cells with RT were 0.66±0.16 %.

With increasing the applied dose, a statistically significant elevation in the incidence of induced RT ($P<0.01$) occurred – up to 3.66±0.72%. The dose of 2.5 Gy induced both ring and chain quadrivalents from the CIV type (Fig. 2), and the proportion of both RT types was about 2:1 in favour of rings.

Reproductive capacity

The data from mating of non-irradiated females with males irradiated at 2.5 Gy at various terms after the irradiation are shown in Table 2.

After irradiation of male rabbits at 2.5 Gy, a reduced fertility rate of mated females was observed: 66.6% by post irradiation days 10 and 30, 75% by post irradiation day 20 and 41.4% by day 40, compared with 83% fertility rate in controls.

The average number of liveborns per doe was lower than in controls and varied from 4.8 to 7.0 in experimental groups, vs 7.5 in controls.

The observed reduction in aforementioned parameters was related to the decreased litter size, the reduction being 20% 30 days after the irradiation and reached a maximum by day 40 – 46%.

The reduction in litter size, observed in the present study, as an indicator of the reproductive capacity of irradiated male rabbits, was similar to results reported in other rodents and mammals (Caine & Lyon, 1979). The observed model of sterility and reduction in litter size in the time interval between the 10^{th} and 40^{th} days after the irradiation was indicative for the effect of radiation upon germ cells in various stages of spermatogenesis and this model supported the hypotheses of Generoso *et al.* (1982), Cattanach *et al.* (1990), that postmeiotic germinative cellular stages of spermatogenesis were more sensitive to the induction of mutations than to cellular death.

Table 1. Incidence of reciprocal translocations in rabbit spermatogonia after irradiation with 0.5 Gy and 2.5 Gy (mean ± SEM)

Dose	Number of animals	Number of metaphases	Metaphases with translocations (%)	Total translocations (%)
0.5 Gy	3	600	0.66±0.16	0.83±0.16
2.5 Gy	3	600	3.50±0.78[a]	3.66±0.72[a]

a - $P< 0.01$ vs the group irradiated at 0.5 Gy.

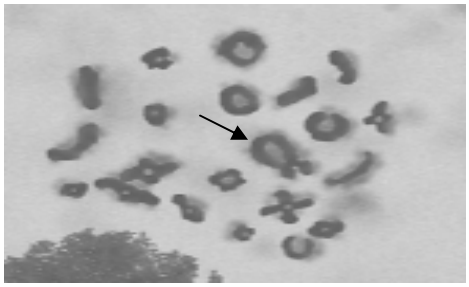

Fig. 1. Spermatogonia of rabbits irradiated at 0.5 Gy: meiotic metaphase I with ring quadrivalent.

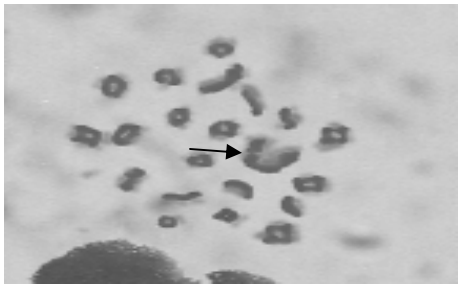

Fig. 2. Spermatogonia of rabbits irradiated at 2.5 Gy: meiotic metaphase I with chain quadrivalent.

Having investigated the mutagenic effect of the ionized radiation upon germinative cells, Lyon (1981) observed a different radiosensitivity of irradiated spermatogonial stem cells and post stem cellular stages. The offsprings of spermatozoa irradiated in the different stages of spermatogenesis differed in various aspects, such as reduction in litter size (probably due to DLM), incidence of partial sterility (due to RT) and cases of full sterility of sons (also probably due to induced translocations). In all these aspects, the descendents of irradiated post stem cellular stages were more severely damaged than those descending from treated spermatogonial stem cells (Generoso *et al.*, 1980).

According to the duration of spermatogenesis cycle in rabbits stated by Desjardins (1972) and Foote (1998), the interval between the 10^{th} and 40^{th} day after the irradiation corresponds to definite stages of spermatogenesis, namely – spermatozoal, early spermatid, late spermatid and late spermatocyte stage. The radiosensitivity model in our study confirmed the data of Russel *et al.* (1990) and showed that late germinative cellular stages were less sensitive than the early ones: early spermatid and late spermatocyte stages.

Table 2. Average number of liveborns per doe and percentage reduction of the litter after mating of non-irradiated ♀ and ♂ irradiated at 2.5 Gy

Parameters	Groups (post irradiation days at mating)				
	IV (10 days)	V (20 days)	VI (30 days)	VII (40 days)	VIII (controls)
Number of does	12	12	12	12	12
Number of pregnant does	8	9	8	5	10
Percentage of pregnant does, %	66.6	75	66.6	41.6	83
Liveborn rabbits	56	63	48	24	75
Number of liveborns per doe	7	7	6	4.8	7.5
% reduction of litter size	6.7	6.7	20	46	

On the other side, the radiosensitivity of spermatogonia as precursors of maturing and mature germinative cells was most important for evaluation of the genetic risk for future generations (Russel *et al.*, 1998). The genetic damage induced in spermatogonia persisted either completely or partially to the end of the reproductive period.

It is hypothesized that the definitive mutation yield in spermatozoid was determined by a complex of factors as the intensity of reparation processes, heterogeneity of spematogonial population with regard to radiosensitivity, selective death of most radiosensitive cells etc. The humped curve that describes the RT yield in several mammalian species (Haines *et al.*, 2002) is probably due to the coincidence of the radiosensitivity of spermatogonia to mutability and to cellular death. It is known that spermatogonia, carriers of translocations, having passed through the meiosis, yield 4 classes of spermatozoa: 2 classes with a non-balanced genome (duplication or deficiencies) 1 class with balanced and 1 – with normal genome. A big part of fertilizations realized by spermatozoa with non-balanced genome yield zygotes that perish in the early stages of their development (prior to the implantation or between implantation and the parturition), i.e. they behave as dominant lethals (Selby, 1990). A small part of zygotes with unbalanced genome could yield a full course of pregnancy and give birth to a healthy progeny, that carries multiple abnormalities (Oakberg, 1984).

CONCLUSIONS

The analysis of our data showed a differentiated radiosensitivity of post meiotic germinative cellular stages of spermatogenesis. The external gamma irradiation causes a reduction in fertility rate and decreased litter size, most apparent by post irradiation day 40.

The RT, induced in irradiated spermatogonia, being among the most important parameters of genetic risk for further generations, are dependent upon the rate of applied dose. They reached 3.66±0.72 % after irradiation at 2.5 Gy – a value, that was significantly higher than that of 0.83±0.16 % after irradiation with 0.5 Gy.

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