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Original Contribution

GENETIC EFFECTS IN SOMATIC AND GERM CELLS IN RABBITS FOLLOWING EXTERNAL GAMMA RADIATION. I. RECIPROCAL TRANSLOCATIONS IN SPERMATOGONIA

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ABSTRACT

Male rabbits, aged 4.5 months, were subjected to graded total gamma irradiations of 0.5 Gy, 1.5 Gy, 2.5 Gy and 3.0 Gy with a dose density of 24 cGy/min. The genetic effects in spermatogonia were assessed 3 months later using cytogenetic analysis of chromosomal preparations.
The data showed a dose-dependent increase in the frequency of reciprocal translocations, induced in spermatogonia and detected in the spermatocyte stage in diakinesis metaphase I. The highest frequency of reciprocal translocatins was observed in rabbits irradiated at 3.0 Gy. The dose-effect function was linear from the type $y=a+\beta D$.

Key words: Gamma irradiation, spermatogenesis, translocations, stem cells.

INTRODUCTION

The classical method of biological dosimetry, detection of radiation-induced chromosome aberrations in peripheral blood lymphocytes, gives only a general notion of the effect of radiation on the gonad. The cytogenetic analysis of lymphocytes is an indicatory model, but could not be used as a model of the potential risk for generations and populations as a whole

The spermatogenesis in male individuals is an exceptionally sensitive in vivo system for biological dosimetry of ionized radiation (1) because of the high sensitivity of germ cells to radiation-induced death and their sensitivity to induction of mutations (2). The available literature data show a considerable heterogeneity in the radiosensitivity of the various cell types during spermatogenesis. Despite the fact that spermatids are considered to be among the populations most susceptible to genetic damage (3), spermatogonia are incontestably the critical component because of their nature; they are the precursors of the next generations of developing and mature germ cells (4).

Up till now the test for detection of translocations in diakinesis metaphase I is used in the study on the mutagenic effect of ionized radiations in mammals. This test evaluates the frequency of induced translocations in spermatogonia and predicts the resulting genetic damage in F1 (5).

The knowledge of the fate of induced translocations in spermatogonia is especially important in the precise evaluation of genetic risk. Genetically impaired spermatogonia from meiosis form spermatozoa with non-balanced, balanced or normal genome. This situation could be responsible for the appearance of dominant lethal mutations, offspring with multiple abnormalities, as well as phenotypically normal, heterozygous translocations in the progeny (6, 7, 8, 9).

The present study aimed to detect the frequency of reciprocal translocations in spermatogonia of rabbits, irradiated with graded doses of gamma rays in order to determine the correlations between the dose and the effect on the genetic makeup.

MATERIAL AND METHODS

The experiment was performed with sexually mature 4.5-month old male New Zealand rabbits. All animals were of equal body

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weight (4.0–4.5 kg) and were housed in individual cages in uniform conditions prior to and during the experiment.

The animals were subjected to total irradiation with ^{60}Co at the dose range of 0.5-3.0 Gy and at dose density of 18 cGy/min, using the gamma equipment (Rokus). The rabbits were divided into 4 groups (n>3), with each group receiving treatment as follows:

- Group I: rabbits irradiated at 0.5 Gy;
- Group II: rabbits irradiated at 1.5 Gy;
- Group III: rabbits irradiated at 2.5 Gy;
- Group IV: rabbits irradiated at 3.0 Gy.

The exposure dose was calculated according to the geometrical parameters of the source, its power and the source-object distance (10).

To determine the reciprocal translocation frequency induced in spermatogonia in the irradiated rabbits three months later, preparations for cytogenetic analysis of spermatocytes in diakinesis metaphase I were done using the method of Evans et al. (11) but modified for the rabbits. This modified method is a combination of separate procedures from the methods of Evans et al. and Schleiermacher (12). The modification affected only the primary processing, i.e. the hypotonic processing of the material and the duration of the procedure. The testis was removed from its envelopes and about half of it was homogenized completely. Then hypotonic processing followed for 8 min using 1.12% sodium citrate solution at 37^{o}C. The modification included separation of the homogenization and hypotonisation stages. It was important that each stage was carried out with precision in order to obtain high-quality preparations with sufficient amount of spermatocytes in diakinesis metaphase I.

An average of 200 metaphase preparations was analysed from each animal supported by the relevant statistical backup.

RESULTS

A total of 2400 cells from 12 rabbits irradiated at 0.5, 1.5, 2.5 and 3.0 Gy was analysed (**Table 1**). The reciprocal translocations in rabbit spermatogonia were detected at the spermatocyte stage in diakinesis metaphase I.

Table 1. Number of reciprocal translocations in spermatocytes, induced in rabbit spermatogonia after total irradiation in the 0.5-3.0 Gy range.

Dose	Number of rabbits	Number of cells scored	Cells with translocations, %	Total translocations, %
0,5 Gy	3	600	0,66±0,16	0,83±0,16
1,5 Gy	3	600	2,33±0,44	2,33±0,44
2,5 Gy	3	600	3,5±0,78	3,66±0,72
3,0 Gy	3	600	4,0±0,5	4,33±0,6

Table 2. Individual results of the analysis of spermatocytes induced in rabbit spermatogonia after total irradiation in the 0.5-3.0 Gy range.

Dose	Metaphases	Number of animals	Cells with translocations	Total number of translocations
0,5 Gy	200	1	1	1
0,5 Gy	200	2	2	2
0,5 Gy	200	3	1	2
	600		4	5
1,5 Gy	200	4	6	6
1,5 Gy	200	5	3	3
1,5 Gy	200	6	5	5
	600		14	14
2,5 Gy	200	7	7	7
2,5 Gy	200	8	4	5
2,5 Gy	200	9	10	10
	600		21	22
3,0 Gy	200	10	7	7

3,0 Gy	200	11	7	8
3,0 Gy	200	12	10	11
	600		24	26

The analysis of data showed that the irradiation at 0.5-3.0 Gy resulted in increased frequency of reciprocal translocations in irradiated spermatogonia.

After irradiation at 0.5 Gy, the percentage of cells with translocations was 0.66±0.16%. In all 600 metaphase preparations of three rabbits, only configurations from the R IV type were observed. (**Figure 1.**)

Figure 1. Meiotic metaphase I showing ring quadrivalents.

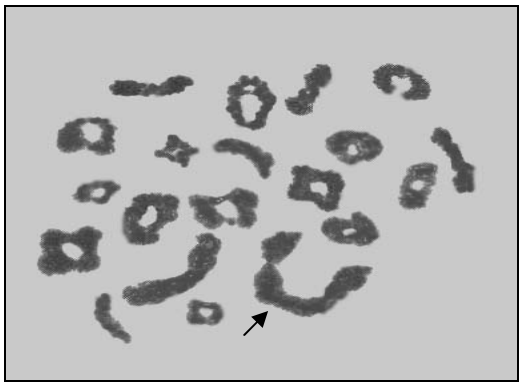

Figure 2. Meiotic metaphase I showing chain quadrivalents.

In 1.5 Gy rabbits, the percentage of cells with reciprocal translocations reached 2.33±0.44% and, apart from the ring configurations, multivalents from the C IV type were also observed.(**Figure 2**) The tentative ratio of both translocation types was 2:1 in favour of the ring type.

The irradiation within the dose range 2.5-3.0 Gy increased the occurrence of cells with reciprocal translocations that reached 3.5±0.86 and 4.0±0.5 per 100 metaphase preparations. In this dose range, the ratio of ring and chain multivalent configurations was almost 1.6:1; there were metaphase preparations with R IV and C IV quadrivalents.

The regression analysis of results, using the equation below, showed a linear correlation between the number of reciprocal translocations and the dose of irradiation:

$$Y = (2.93 \pm 021).D, p<0.001,$$

where Y= number of reciprocal translocations and D = dose of irradiation.

Our results showed a linear increase in the number of reciprocal translocations in spermatogonia of irradiated rabbits within the dose range 0.5-3.0 Gy. The highest percentage (4.3%) was observed in the 3.0 Gy group. The studies in the field of radiation mutagenesis with other species always reported a hump dose-effect curve. The peak of reciprocal translocations in mice was at 6.0-7.0 Gy (13), in guinea pigs – at about 3.0 Gy (14), in men and marmosettes – at the much lower dose of 1.0 Gy (15). The hump dose-effect curve could be explained by the complexity of the spermatogonial cell population and the heterogeneity in its sensitivity to mutations from one part and to cellular death from the other. According to Lion et al. (14) the peak in translocations is achieved when the degree of translocation production is balanced by the degree of germinative cells elimination as a result of cellular death. The inter-species differences in the production of translocations and cellular death at various doses determined the various site of the peak of reciprocal translocations.

Our data are in accordance with those of Lion et al. (14) in rabbits, although we did not perform experiments with doses higher than 3.0 Gy.

Both our data and the analysis of literature data showed that in rabbits, the sensitivity to induction of reciprocal translocations was lower than that in mice possibly because of the lower sensitivity of all cells from the radiosensitive population or the lower sensitivity of this part of the population susceptible to genetic damage.

In experiments with postmeiotic germinative cell stages, especially spermatozoa, Cox et al. (16) put species depending on their sensitivity to induction of

reciprocal translocations in the following order: mouse, rabbit, guinea pig and hamster. With regard to the evaluation of the risk for the appearance of mutations in human germinative cells in conditions of over-background radiation exposure, the question whether the damage in one cellular type could be extrapolated to another one is imperative.

The observed resemblance in various species requires a particular attention in data interpretation, especially for data on spermatozoa because of the specificity of this population and the fact that genetic damages are directly transferred to the zygote without being selected or removed during mitosis, unlike genetic damages induced in the early stages of spermatogenesis.

CONCLUSIONS

Our results showed a dose-dependent increase in the occurrence of reciprocal translocations, induced in rabbit spermatogonia within the 0.5-3.0 Gy range. The percentage of ring quadrivalents was higher than that of chain ones. The dose-effect relationship was described by a linear function.

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