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Dominant lethal effect of ^{60}Co gamma radiation in *Biomphalaria glabrata* (SAY, 1818)

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Abstract

The dominant lethal effects of gamma radiation of ^{60}Co in the snail *Biomphalaria glabrata* were studied. Three groups of 13 wild-type snails were irradiated with single doses of 2.5; 10 and 20 Gy. Crossings were carried out at intervals of 7, 17, 23, 30 and 36 days after irradiation. The dominant lethal effect was observed only at the first crossing occurring 7 days after irradiation with 2.5 Gy. With 10 and 20 Gy, the induction of lethal mutations was detected at 7, 17 and 23 days after irradiation; a dose-response effect was observed. The effect was stronger 7 days after irradiation, decreasing in the succeeding crossings up to 30 days. Cell-killing effects on germ cells were detected in the crossings at 23 days and 30 days after irradiation with 20 Gy. After 36 days, frequencies of malformations resumed background levels; crossing rates partially recovered. These results show that gamma radiation affected all the stages of spermatogenesis. Germ cells at later phases were more sensitive to the mutagenic effect of radiation and the cell killing effects were observed on the youngest cells. This response was similar to the highly homogeneous pattern observed in widely different species and allowed us to estimate some parameters of spermatogenesis in *B. glabrata*.

Keywords: Dominant lethal test; *Biomphalaria glabrata*; Freshwater snail; Ionizing radiation; Embryonic malformations; Biomonitoring

1. Introduction

Among the effects of mutagenic pollutants on ecosystems, the induction of germ cell mutations causes the most severe consequences since they can directly affect the reproductive potential of populations [1]. Nevertheless, there are few studies on germ cell mutagenicity of pollutants in natural populations.

We have established the dominant lethal test in the freshwater mollusk *Biomphalaria glabrata* [2]. This snail is a good experimental model for environmental biomonitoring studies due to its ecological importance and biological characteristics. Invertebrates represent more than 90% of aquatic species and among mollusks, 80% correspond to gastropods [3,4]. The genus

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Biomphalaria shows wide geographical distribution and is represented in Brazil by 10 species [5]. It has low dispersion and is easily collected. Other aspects make this system a good model for laboratory studies. The snails are easy to breed, need little space, can reproduce throughout the year under controlled conditions and have a short life-span: an egg-to-egg monitoring can be done in two months in *B. glabrata*. The dominant lethal test was established in *B. glabrata* with mitomycin *c* and cyclophosphamide [2]. This system was efficient, specific and sensitive in the evaluation of germ cell mutations induced by reference mutagens. Positive results obtained with both compounds showed that *B. glabrata* can absorb and activate chemical mutagens from the aquatic environment.

The validation of a mutagenicity test needs an evaluation of its performance when submitted to different reference agents. Ionizing radiation acts as a direct mutagen with minimal latency time of action, since it does not require absorption, distribution or metabolism; furthermore, the time of effective activity is practically zero. Thus, ionizing radiation can be used as reference to infer some kinetic parameters of chemical mutagens, considering that, sometimes, the correlation between the pharmacokinetics of chemicals and their genotoxic activity is complex. It is particularly useful when the agent requires metabolic activation, its mechanism of action is ignored, or its effective time of action is very short or very long [6]. The effects of ionizing radiation on germ cells are well known. The aim of this work was to evaluate the effects of ^{60}Co gamma radiation in the dominant lethal test in *B. glabrata*.

2. Material and methods

2.1. Animals

A pigmented wild-type strain of *B. glabrata* obtained from Barreiro (state of Minas Gerais, Brasil) and a non-pigmented albino strain, originating from Amaralina (state of Bahia, Brasil), reared in the laboratory over the past 14 years were used. The homozygosity of the wild-type snails was assumed based on the long period they were kept in inbreeding conditions; this was later confirmed on basis of the results of the crossings.

The colonies are maintained in plastic aquaria with filtered, dechlorinated and aerated water and fed fresh lettuce. For the experiments, animals were isolated for at least three weeks before the onset of the experiments in individual aquaria under the same conditions as the colonies. We used sexually mature snails at least two months old and with a minimal shell diameter of 10 mm.

2.2. Animal selection

All snails used in the experiments were selected by previous analysis of background frequencies of embryonic malformations. Three or four egg-masses were collected from albino snails before the crossings and those with malformation frequencies above 5% were discarded. Wild-type snails were evaluated after a crossing with albino snails before the exposure. At least 50 phenotypically wild-type embryos per animal were analyzed among the progeny of the albino snails and those with frequencies of malformations above 3% were discarded.

2.3. Exposure and crossings

Wild-type snails were exposed to ionizing radiation in plastic tubes, individually separated by cotton pads. The irradiation was carried out in a ^{60}Co source—Gamma-Cell Atomic Energy of Canada LTD, model GC-220 from Instituto de Pesquisas Energéticas e Nucleares/Comissão Nacional de Energia Nuclear, in the presence of molecular oxigen at room temperature. Each wild-type snail was paired with a non-exposed albino snail for 24 h in individual aquaria at several intervals after irradiation. Afterwards, animals were isolated and egg capsules were collected from albino snails.

Three groups of 13 wild-type snails were irradiated with single doses of 2.5, 10 and 20 Gy, at a dose-rate of 0.40 Gy/min; 13 animals served as controls. Crossings were carried out at intervals of 7, 17, 23, 30 and 36 days after irradiation.

2.4. Analysis of embryonic malformations

Egg capsules were collected daily. Plastic sheets were placed on the water surface to propitiate oviposition and egg capsules were then transferred to cell

culture plates and maintained in climatic chambers at 25 °C until the end of the analysis. After a crossing between homozygous wild-type snails and albino snails, both groups of animals produce embryos; for this study, only the offspring of the albino snails was analysed. Among the offspring of the albino snails, the following types of embryos are produced: (i) heterozygous wild-type embryos produced by cross-fertilization and (ii) albino embryos produced by self-fertilization or by cross-fertilization with other albino snails before the onset of the experiment. Phenotypically wild-type embryos can be identified among the offspring of albino snails by the presence of pigmentation in the eyes, visible from the fourth day on of embryonic development. Thus, we analyzed the wild-type embryos, originated from sperm of the exposed wild-type snails; albino embryos were discarded.

Embryos were observed for 8 days from the beginning of the development until nearly hatching using a stereomicroscope (Olympus). The number of teratomorphic or hydropic wild-type embryos—in which multiple structures are affected—was scored among the offspring of albino snails. These embryos are included in the category of unspecific malformations according to Geilenkirchen [7].

All analyses were carried out in coded scoring.

2.5. Statistical analysis

To compare the malformation frequencies among the different groups, the χ^2-test for Poisson distributions was used. The Poisson model was considered since it has a good adjustment in presence of rare events. To compare the crossing rates among the different groups the χ^2-test for homogeneity was used.

3. Results

Results of the experiments are shown on Table 1 and Fig. 1.

Table 1
B. glabrata dominant lethal mutation test of ^{60}Co gamma radiation

Crossing schedule (days)	Dose (Gy)	No. of pairings	No. of crossings	Wild-type embryos	
				Total	Malformed (%)
7	0	11	10	2316	12 (0.52)
	2.5	11	11	2108	42 (1.99)**
	10	11	11	1737	377 (21.70)**
	20	13	12	1825	920 (50.41)**
17	0	11	10	2276	2 (0.09)
	2.5	12	11	2207	8 (0.36)
	10	10	10	2176	115 (5.28)**
	20	12	12	1874	284 (15.15)**
23	0	10	10	2267	13 (0.57)
	2.5	12	12	2016	21 (1.04)
	10	11	11	1267	37 (2.92)**
	20	12	7*	809	94 (11.62)**
30	0	13	13	2151	5 (0.23)
	2.5	12	11	2398	5 (0.21)
	10	9	8	839	1 (0.12)
	20	11	0**	–	–
36	0	13	12	1779	4 (0.22)
	2,5	12	12	1838	3 (0.16)
	10	11	8	1302	3 (0.23)
	20	12	4*	578	1 (0.12)

* $P < 0.02$.
** $P < 0.0001$.

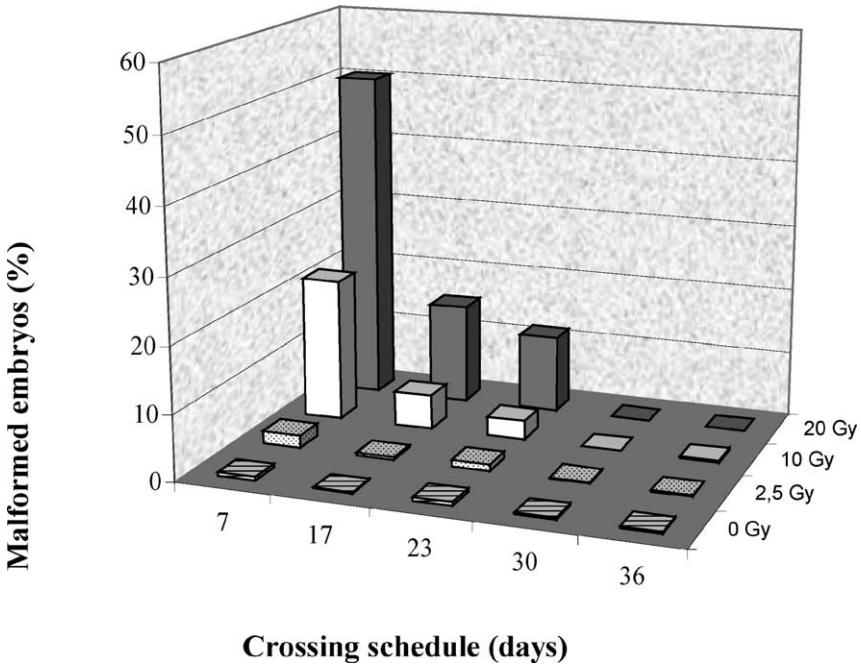

Fig. 1. Frequencies of malformation in the wild-type offspring of albino snails crossed with the wild-type snails at different intervals after the exposure to ^{60}Co gamma radiation.

In all control groups, the frequencies of non-viable embryos, which correspond to the total of dead and malformed embryos, were under 5%, established as the background level to control groups in experiments with embryos of *B. glabrata*.

Selection of the doses and post-irradiation crossings were based on a preliminary experiment, in which two groups of five snails were irradiated with single doses of 2.5 and 30 Gy, at a dose-rate of 0.43 Gy/min; five animals served as controls. Wild-type snails were paired with albino snails at intervals of 10, 20, 25, 30, 35, 40, 45 and 50 days after irradiation. Increases in the frequencies of malformations were observed only at the first crossing occurring 10 days after irradiation with 2.5 Gy; with 30 Gy, this effect was detected 10 and 20 days after irradiation. A dose-response effect was observed. A reduction in the crossing rates was detected 20–30 days after irradiation with 30 Gy; with 2.5 Gy this effect was not observed. After 35 days, both frequencies of malformations and crossing rates resumed background levels. Thus, a dose range of 2.5–20 Gy and a crossing interval of 7–36 days after irradiation were used.

In our system, the induction of dominant lethal mutations leads to an increase in the frequency of unspecific malformations and cell-killing effects are evidenced by a reduction in the crossing rates. Thus, the dominant lethal effect was evaluated by comparing the frequencies of malformations in the heterozygous offspring of the albino snails crossed with the wild-type snails at the different intervals after irradiation. To evaluate the effects on crossing rates, numbers of effective crossings, that is, albino snails that produced heterozygous embryos, were compared to the numbers of pairings among the different groups.

The effects of ionizing radiation were observed at the three doses (Table 1). The dominant lethal effect was observed only at the first crossing occurring 7 days after irradiation with 2.5 Gy. With 10 and 20 Gy, the induction of lethal mutations was detected at 7, 17 and 23 days after irradiation; a dose-response effect was observed. The effect was stronger 7 days after irradiation, decreasing in the succeeding crossings up to 30 days. From that time on, no induction of lethal mutations was observed. Cell-killing effects were detected in the crossings at 23 days and 30 days after

irradiation with 20 Gy. After 36 days, crossing rates began to recover but control levels were not reached.

4. Discussion

After establishing the dominant lethal test in *B. glabrata* using two reference chemical mutagens—mitomycin *c* and cyclophosphamide [2]—we decided to use ionizing radiation as another reference agent.

The induction of dominant lethal mutations by chemical mutagens in spermatogenesis is stage-specific; ionizing radiation, by contrast, affects germ cells at all stages. Each developmental stage, however, responds with a different sensitivity [8].

In this study, a variation in the radiosensitivity among spermatogenic cells at the different developmental stages was observed. Germ cells at later phases of spermatogenesis were more sensitive to the induction of dominant lethal mutations than those at earlier stages. The maximum increase in the frequencies of malformations 7 days after irradiation showed that mature spermatogenic cells were the most sensitive to the mutagenic effect of ionizing radiation. On the other hand, the reduction on the crossing rates 23 and 30 days after irradiation showed that the killing effects of gamma irradiation were greater on the youngest germ cells.

The duration of spermiogenesis in *B. gIabrata* is about two weeks [9] and there is no further information on the chronology of spermatogenesis in this species. Therefore, the first crossing at 7 days after exposure evaluated the effect of ionizing radiation on the germ cells at spermatozoa/spermatid stage.

The complete chronology of spermatogenesis in *Biomphalaria* has not been established, thus it was not possible to determine all the affected stages. Although we have not performed histological preparations of gonads, it was possible, based on the results of the dominant lethal test obtained in this study, to make an estimate of the timing of spermatogenesis in *B. glabrata*.

The radiosensitivity of germ cells has already been studied in several systems. In general, pre-meiotic germ cells are less sensitive to the induction of dominant lethal mutations than post-meiotic cells and highly sensitive to the cell-killing effect. These effects can be attributed to two factors: (i) young mitotically active gametogenic cells would have a greater capacity to repair sub-lethal DNA damage that produces dominant lethality and (ii) there would be a selective elimination of the damaged cells [10,11]. A striking uniformity in response between germ cells at homologous stages of spermatogenesis in widely different species was observed, which underlines the evolutionary constancy of the process of meiosis [10].

The high sensitivity of spermatogonia to the cell killing effect of ionizing radiation was observed in mammals [12–14], fish [15], molluscs [16] and insects [17,18].

In our study, cell-killing effects were observed 23 and 30 days after irradiation with the highest dose. These results suggest that spermatogenic cells evaluated at these crossings were exposed at the spermatogonia stage.

A recovery in the frequencies of malformations back to the control levels was observed after 36 days. In the preliminary study, malformation and crossing rates resumed background levels after 35 days, even with a high dose of 30 Gy, which suggests that these gametes originated from a new pool of cells, not affected by radiation. Thus, the duration of the spermatogenesis en *B. glabrata* was estimated to be approximately 36 days.

In the freshwater snail *Lymnaea stagnalis*, the exposure to 10,000r of gamma radiation damaged all stages of gametogenesis and caused a temporary sterile period. In about 30 days, the empty acini were repopulated with new eggs and sperm [19].

In our study, crossing rates partially recovered after 36 days but control levels were not reached in the dominant lethal test as observed in the preliminary experiment with ionizing radiation.

An asynchrony in the development of spermatogenic cells could be responsible for the discrepancy between the preliminary experiment and the results of this test. Tuan and Simões [20] observed that the meiotic process in *B. tenagophila* is slower down by dessication. Egami and Hyodo-Taguchi [21] found a difference of 13 days in the total duration of spermatogenesis in the fish *Oryzias latipes* kept at 25 °C or at 15 °C.

From the analysis of dominant lethal and cell killing effects of ^{60}Co gamma radiation in *B. glabrata* and considering the conservative pattern of response of

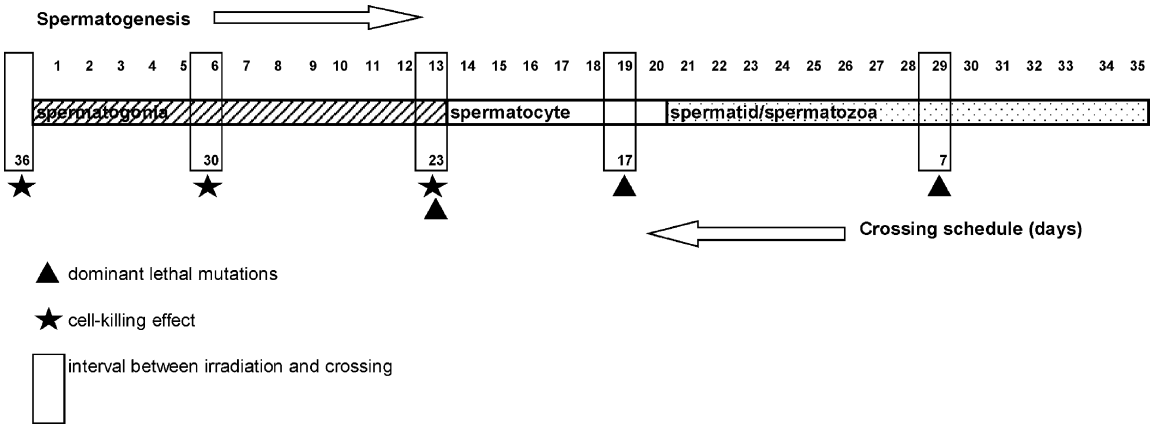

Fig. 2. Estimated chronology of spermatogenesis in *B. glabrata*.

spermatogenesis to ionizing radiation, we made an estimate of the chronology of spermatogenesis in this species (Fig. 2).

Total duration of spermatogenesis was estimated to be approximately 36 days, with the following distribution of developmental stages: 1–13 days, spermatogonia, 14–20 days, spermatocytes, 21–36 days, spermatids and spermatozoa.

The purpose of the post-exposure crossings in the establishment of the dominant lethal test in *B. glabrata* with chemical mutagens [2] was solely to confirm the genetic nature of the induced malformations; additional crossings were not done because of the lack of information on the chronology of spermatogenesis in gastropods. Further studies on the time course of spermatogenesis by autoradiographic techniques should be carried out to determine the affected stages.

The data obtained in this work by studying the effects of ^{60}Co gamma radiation will be useful as a reference for the choice of post-exposure crossings in the dominant lethal test with other agents. They will also be helpful for the interpretation of data obtained from the analysis of environmental samples, usually complex mixtures of many chemicals.

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