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The use of a micronucleus test to characterise adaptation of *Vicia faba* root tip cells to gamma-radiation

P. KUGLÍK and R. VESELSKÁ

Department of Genetics and Molecular Biology, Faculty of Sciences, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic

Abstract

Pretreatment of *Vicia faba* root tip cells with low doses of ^{60}Co γ-radiation can reduce the yield of micronuclei induced by a subsequent high dose of γ-radiation (1.5 Gy). This adaptation to γ-radiation possesses the following characteristics: (*1*) this phenomenon can be induced by an optimal range of low doses of γ-radiation applied acutely or fractionally; (*2*) the expression of this response seems to be transitory and depends on the time interval between adapting and challenging doses of γ-radiation.

Introduction

"Radio-adaptive response" is the term used to describe protection of the higher eukaryotes by low doses of ionizing radiation against the effects of subsequent high doses of radiation (Wolf *et al.* 1988, Ikushima 1989). Although very little is known about the mechanisms underlying the adaptive response to ionizing radiation, there are hypotheses that the low doses protect the cells against subsequent radiation, possibly by the induction of protein enzyme repair system (Wiencke *et al.* 1986).

In our previous experiments (Kuglík and Šlotová 1991), we found that internal β-irradiation from incorporated tritiated thymidine can protect *Vicia faba* root tip cells against subsequent γ-irradiation when assayed by the induction of micronuclei.

In the present paper, we investigated the effects of external ^{60}Co γ-irradiation at low doses. The purpose of these experiments was to find whether the "radio-adaptive response" could be induced in *Vicia faba* root tip cells if the priming dose is given acutely or fractionally, and whether the time between the low and high dose exposures required for optimal expression of the response could be determined.

Materials and methods

The experimental material consisted of primary root tip meristems of *Vicia faba* cv.

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Inovec (2n=12). The method of cultivation has been described elsewhere (Kihlman 1975). Seedlings with primary roots 3 to 4 cm long were used for the experiments.

The irradiation was carried out using a cobalt source (*Chisostat*) at a dose rate of 0.1 Gy min^{-1} for low-dose exposure (0.01 - 0.09 Gy) and 0.39 Gy min^{-1} for high-dose exposure (1.5 Gy). The seedlings were irradiated on moistened filter paper at room temperature. After irradiation, the seedlings were transferred to a water tank containing fresh, aerated tap water (20 °C).

For scoring micronuclei, the root tips were taken 24 h after the challenge irradiation with a dose of 1.5 Gy, when most of the cells had entered the second cell cycle. The root tips were then fixed in cold ethanol-glacial acetic acid (3:1), hydrolysed 10 min in 1 M HCl (60 °C) and the slides Feulgen stained. The incidence of micronuclei in the root tip cells of *Vicia faba* was determined based on an evaluation of 15 000 cells per treatment (1 000 cells per tip) and all the results were statistically evaluated using the Mann-Whitney test.

Results

Adaptations induced in *Vicia faba* cells by acute low doses of γ-radiation: Meristematic cells of *Vicia faba* were pre-irradiated with a priming low dose of γ-radiation (0.01 - 0.09 Gy) and, 2 h later, with a challenge dose of 1.5 Gy. The acute pre-irradiation with the low dose of γ-radiation induced a moderate but significant increase in the

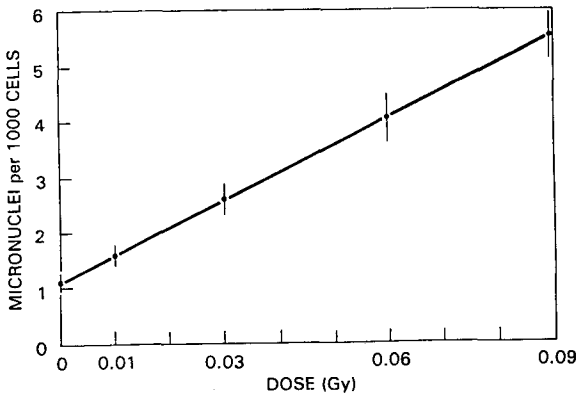

Fig. 1. Induction of micronuclei in *Vicia faba* root tip cells by acute irradiation with priming doses of 0.01 - 0.09 Gy (mean ± S.E.).

number of micronuclei (Fig. 1). In some cases, acute pre-irradiation with low doses of γ-radiation can decrease the yield of micronuclei evaluated 24 h after challenging irradiation. The adaptation was strongly dependent on the magnitude of the priming dose of γ-radiation used (Fig. 2). In our experiments, the maximal effect was found after pre-irradiation of meristematic cells of *Vicia faba* with the dose of 0.06 Gy.

Adaptations induced by fractional low doses of γ-irradiation. In these experiments meristematic cells of *Vicia faba* were pre-irradiated with total doses of 0.01-0.04 Gy divided into 1 - 4 fractions of 0.01 Gy each. The time-interval between repeated irradiation lasted 2 h. The irradiation with the challenge dose (1.5 Gy) was carried out as in previous experiments.

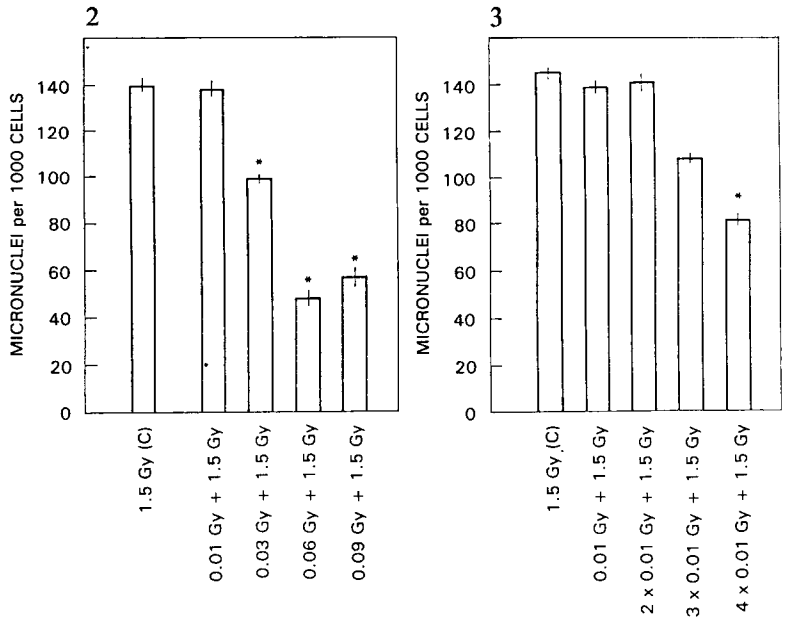

Fig. 2. Effect of acute irradiation with priming doses of 0.01 - 0.09 Gy on the induction of micronuclei by a subsequent challenge dose of 1.5 Gy in *Vicia faba* root tip cells (mean ± S.E.).
* - significantly different from control at $P = 0.05$.

Fig. 3. Effect of repeated irradiation with a priming dose of 0.01 Gy on the induction of micronuclei by a subsequent challenge dose of 1.5 Gy in *Vicia faba* root tip cells (mean ± S.E.)
* - significantly different from control at $P = 0.05$

When a 0.01 - 0.03 Gy priming dose applied fractionally was followed by a 1.5 Gy challenge dose, the number of micronuclei in meristematic cells of *Vicia faba* was lower, but not significantly different from the expected yield. However, *Vicia faba* root tip cells receiving priming dose of 0.04 Gy delivered in four fractions had significantly fewer micronuclei than expected. Thus, it can be seen that repeated pre-irradiation with very low dose is capable of inducing the adaptive response even in the case of using priming dose which is not able to evoke this response after single acute irradiation.

Adaptations in *Vicia faba* cells in dependence on the time interval between priming and challenge γ-irradiation. Meristematic cells of *Vicia faba* were irradiated with a priming dose (0.06 Gy) and a challenge dose (1.5 Gy) separated by intervals of

2 - 24 h. Statistically significant reduction of micronuclei was found only when the two irradiations were separated by an interval of 2 - 6 h (Fig. 4). When they were separated by intervals of 8 - 16 h no adaptation occurred. Thus, the protective effect of a priming dose seems to be transitory and expires when the time interval between two irradiations exceeds a certain limit.

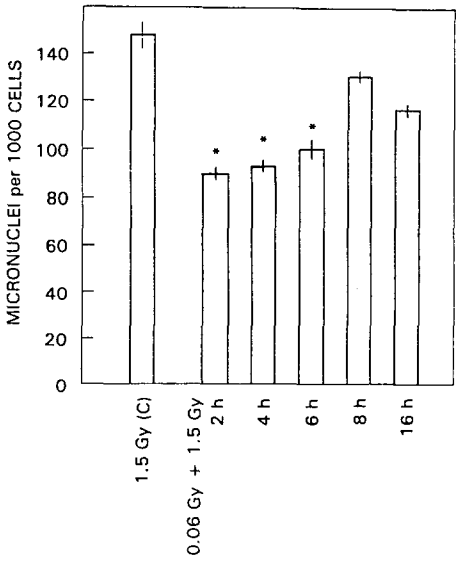

Fig. 4. The incidence of micronuclei in *Vicia faba* root tip cells after acute irradiation with a priming dose of 0.06 Gy and a subsequent challenge dose of 1.5 Gy as a function of the time interval between the irradiations (mean ± S.E.). ** - significantly different from control at $P = 0.01$.

Discussion

Micronuclei represent chromosome fragments or whole chromosomes which are not incorporated into the main nuclei at mitosis. As was described previously (Marshall and Bianchi 1983, Rizzoni *et al.* 1987, Kuglík *et al.* 1990), the induction of micronuclei can be utilised as a very sensitive indicator of radiation-induced chromosomal damage in *Vicia faba* root tip cells.

Our results presented here demonstrate that pre-irradiation with low doses of γ-radiation can decrease the yield of micronuclei induced by subsequent irradiation with the dose of 1.5 Gy. Due to the fact that the low doses used in our experiments did not influence the mitotic index of the pre-irradiated cells (unpublished results), it does not seem probable that the reduced frequency of micronuclei is an artefact caused by the slowing of the cell cycle. An alternative, more likely interpretation of the data is that this cellular phenomenon represents a manifestation of cytogenetic "radio-adaptive response" in *Vicia faba* root tip cells.

Our results confirm that induction of a "radio-adaptive response" in *Vicia faba* root tip cells depends on the magnitude of the low dose used as a primer. Acute pre-irradiation with a priming low dose above 0.03 Gy can induce adaptation in root tip cells, with a maximum at 0.06 Gy. In the case of repeated pre-irradiations, four fractions of 0.01 Gy priming doses were necessary to reduce the yield of subsequent 1.5 Gy-induced micronuclei. These findings are in agreement with the results of previous experiments where optimal dose ranges of both external and internal priming irradiations were found in human lymphocytes (Cai and Liu 1990), Chinese hamster cell (Ikushima 1987, 1989) and *Vicia faba* root tip cells (Heindorff *et al.* 1987a, Kuglík and Šlotová 1991). In addition, Shadley and Wiencke (1989) found that adaptations are dependent both on the total dose of the priming irradiation and on the dose rate at which it is given. The authors conclude that a given number of radiation-induced lesions of DNA produced within certain time span could serve as an inducing signal for the "radio-adaptive response".

An optimal adapting dose has also been found in clastogenic adaptation studies (reduction in yields of chromosomal aberrations after pretreatment with low mutagen doses) induced by certain chemical mutagens such as alkylating agents or maleic hydrazide in *Vicia faba* cells (Rieger *et al.* 1984, Heindorff *et al.* 1987b).

The adaptation of *Vicia faba* root tip cells was a time-dependent process. The maximal reduction in the yield of micronuclei induced by 1.5 Gy, approximately 40 %, was found with a 2 h interval, whereas with intervals of 8 and 16 h only a 10 % reduction was observed. This is consistent with the results observed in human lymphocytes where Wang *et al.* (1991) found that the expression of the cytogenetic adaptive response was transitory with a maximum at 5 h, and disappeared 9 h after the initial low-dose exposure. However, in human lymphocytes this adaptation persisted for about 40 h when assayed by the induction of chromosomal aberrations (Shadley *et al.* 1987). In our experiments, we failed to observe significantly reduced frequency of micronuclei 8 - 16 h after the priming dose. The possibility may be that micronucleus test system is not able to detect this response with longer intervals between priming and challenge doses owing to the dilution of the cells with micronuclei as they divided over the subsequent cell cycle.

In conclusion, the results of this study confirmed that *Vicia faba* root tip cells pre-irradiated with external low-doses of γ-radiation can become less sensitive to the chromosome-breaking effects of subsequent exposure. Evaluation of micronuclei showed that this cellular function depends strongly on the characterisation of the priming radiation dose and the time interval between priming and challenge irradiation.

References

Cai, L., Liu, S.Z.: Cytogenetic adaptive response of somatic and germ cells *in vivo* and *in vitro*. - Int. J. Radiat. Biol. **58**: 35-54, 1990.

Heindorff, K., Rieger, R., Michaelis, A., Takehisa, S.: Clastogenic adaptation triggered by S-phase independent clastogens in *Vicia faba*. - Mutat. Res. **190**: 131-135, 1987a.

Heindorff, K., Rieger, R., Schubert, I., Michaelis, A., Aurich, O.: Clastogenic adaptation of plant cells-reduction of the yield of clastogen-induced chromatid aberrations by various pretreatment procedures. - Mutat. Res. **181**: 157-171, 1987b.

Ikushima, T.: Chromosomal response to ionizing radiation reminiscent of an adaptive response in cultured Chinese hamster cells. - Mutat. Res. **180**: 215-221, 1987.

Ikushima T.: Radio-adaptive response: characterisation of a cytogenetic repair induced by low-level ionizing radiation in cultured Chinese hamster cells. - Mutat. Res. **227**: 241-224, 1989.

Kihlman, B.A.: Root tips of *Vicia faba* for the study of the induction of chromosomal aberrations. - Mutat. Res. **31**: 401-412, 1975.

Kuglík, P., Šlotová, J., Dobřická, R., Karpfel, Z.: *Vicia faba* chromosome damage induced by low doses of gamma radiation. - Biol. Plant. **32**: 113-118, 1990.

Kuglík, P., Šlotová, J.: Different effects of pretreatment with tritiated thymidine on radiation-induced sister chromatid exchanges and micronuclei in *Vicia faba* root tip cells. - Biol. Plant. **33**: 291-297, 1991.

Marshall, I., Bianchi, M.: Micronucleus induction in *Vicia faba* roots, Part I. Absence of dose-rate, fractionation and oxygen effect at low doses of low-LET radiation. - Int. J. Radiat. Biol. **44**: 151-162, 1983.

Rieger, R., Michaelis, A., Nicoloff, H.: "Clastogenic adaptations" of the *Vicia faba* root-tip meristem as affected by various treatment parameters. - Mutat. Res. **140**: 99-102, 1984.

Rizzoni, M., Vitagliano, E., Marconi, M.C., Sottili, A., Gustavino, B.: Micronucleus induction by low doses of X-radiation in *Vicia faba* root tips. - Mutat. Res. **176**: 205-209, 1987.

Shadley, J.D., Afzal, V., Wolff, S.: Characterisation of the adaptive response to ionizing radiation induced by low doses of X-radiation to human lymphocytes. - Radiat. Res. **111**: 511-518, 1987.

Shadley, J.D., Wiencke, J.K.: Induction of the adaptive response is dependent on radiation intensity. - Int. J. Radiat. Biol. **56**: 107-118, 1989.

Wang, Z.Q., Saigusa, S., Sasaki, M.S.: Adaptive response to chromosome damage in cultured human lymphocytes primed with low doses of X-radiation. - Mutat. Res. **246**: 179-186, 1991.

Wiencke, J.K., Afzal, V., Olivieri, G., Wolff, S.: Evidence that [^{3}H]thymidine-induced adaptive response of human lymphocytes to subsequent doses of X-radiation involves the induction of a chromosomal repair mechanism. - Mutagenesis **1**: 375-380, 1986.

Wolff, S., Afzal, V., Wiencke, J.K., Oliveiri, G., Michaeli, A.: Human lymphocytes exposed to low doses of ionizing radiation become refractory to high doses of radiation as well as to chemical mutagens that induce double-strand breaks in DNA. - Int. J. Radiat. Biol. **53**: 39-48, 1988.

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