BIOLOGIA PLANTARUM (PRAHA)

33 (4) : 291–297, 1991

Different Effects of Pretreatment with Tritiated Thymidine (^{3}H–dTh) on Radiation-Induced Sister Chromatid Exchanges (SCEs) and Micronuclei in *Vicia faba* Root Tip Cells

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Abstract. Primary roots of *Vicia faba* were grown for 24 h in the presence of tritiated thymidine (1.85–18.5 kBq ml^{-1}) and then irradiated with a dose of 1.5 Gy of ^{60}Co-gamma- rays. The aim of these experiments was to determine whether low-level endogenous beta-irradiation from incorporated radioactive thymidine could influence the frequencies of sister chromatid exchanges (SCEs) and the numbers of micronuclei induced by subsequent external irradiation with high doses of gamma-rays. The results demonstrated that the pretreatment with ^{3}H-dTh had no significant effect on the frequencies of SCEs in gamma-irradiated root tip cells of *Vicia faba*. In contrast to SCEs, the yields of micronuclei in the ^{3}H-dTh pretreated cells were altogether less than the yield induced by gamma-rays alone (protective effects).

During the last years it has been shown that cultured animal cells exposed to low doses of ionizing radiation from incorporated tritiated thymidine (Olivieri *et al.* 1984) or to low doses of X-rays (Shadley and Wolff 1987, Sankaranarayanan *et al.* 1989) become less susceptible to induction of chromosomal aberrations by subsequent high doses of radiation. This observation has been interpreted as the result of induction of a chromosomal repair mechanism that can decrease the level of chromosomal damage produced by ionizing radiation (Wiencke *et al.* 1986). Thus, low doses of ionizing radiation appear to induce an effect similar to the adaptive response observed with alkylating agents in bacterial (Samson and Cairns 1977), animal (Samson and Schwartz 1980) and plant cells (Rieger *et al.* 1982).

In plants, the inducible responses leading to protective effects at cellular level were observed in unequally fractionally irradiated cells (Sybenga and Kleijer 1976, Leenhouts *et al.* 1982). Recently, clastogenic adaptation to ionizing radiation has been described in *Vicia faba* root tip cells after conditioning pretreatment with low doses of X-rays (Heindorff *et al.* 1987a). The aim of the

Received June 1, 1990; accepted August 27, 1990

present study was to test the effects of the pretreatment with low doses of endogenous beta-radiation from incorporated tritiated thymidine on the formation of SCEs and micronuclei in gamma-irradiated *Vicia faba* root tip cells.

MATERIALS AND METHODS

The experimental material consisted of primary root tip meristems of *Vicia faba,* cv. Inovec (2n = 12). The method of cultivation has been described elsewhere (Kihlman and Andersson 1984). Seedlings with primary roots 3 to 4 cm long were used for the experiments.

The germinated beans were grown for 24 h in a solution containing ^{3}H-dTh (1.85–18.5 kBq ml^{-1}, spec. activity 910 GBq mmol^{-1}), which acted as a source of low-level beta-radiation. The external gamma-irradiation was carried out using a cobalt source (Chisostat) at a dose rate of 0.38 Gy min^{-1}. The seedlings were irradiated with a dose of 1.5 Gy on moistened filter paper at room temperature.

For SCE detection, the seedlings after irradiation were grown at 20 °C in the dark at first for 17 h in a solution of 100 μM BrdU + 0.1 μM FdU + 5 μM Urd, and then for 21 h in a solution of 100 μM dTh + 5 μM Urd. To obtain a sufficient number of chromosomes in metaphase, the roots were exposed for the last 2 h to 0.05% colchicine. After fixation in methanol-glacial acetic acid (3:1) for 24 h, the FPG technique (fluorescence plus Giemsa) was performed as described by Cortés and Andersson (1987). The frequencies of SCEs were determined basing on the evaluation of 480 chromosomes (40 cells) of the standard karyotype of *Vicia faba.*

For scoring micronuclei, the root tips were taken 24 h after irradiation, when most of the cells had entered the second cell cycle. The root tips were then fixed and the slides were Feulgen stained. The incidence of micronuclei in the irradiated cells of *Vicia faba* was determined basing on the evaluation of 10 000 cells and all the results were statistically evaluated using the Student *t*-test.

RESULTS

The yields of SCEs and micronuclei induced by treatment with ^{3}H-dTh alone

In the first group of experiments, we monitored the rate of radiation damage to chromosomes induced by low-level chronic beta-irradiation from ^{3}H-dTh alone. As follows from Fig. 1, the treatment for 24 h with ^{3}H-dTh (1.85–18.5 kBq ml^{-1}) did not affect significantly the frequencies of SCEs when compared with control values. In contrast to SCEs, the yields of micronuclei induced by ^{3}H-dTh alone increased linearly with the increase in concentrations of ^{3}H-dTh

Abbrevations: BrdU = 5-bromo-2 -deoxyuridine, dTh = thymidine, FdU = 5-fluoro-2-deoxyuridine, FPG = fluorescence plus Giemsa, SCE = sister chromatid exchange, Urd = uridine.

within the whole dose range used in our experiments. It should be mentioned that concentrations of the isotopes used for the pretreatment were determined in preliminary experiments and were so chosen as to give yields of micronuclei comparable to those observed after irradiation with low doses (0.01–0.20 Gy) of gamma-rays (Kuglík *et al.* 1990).

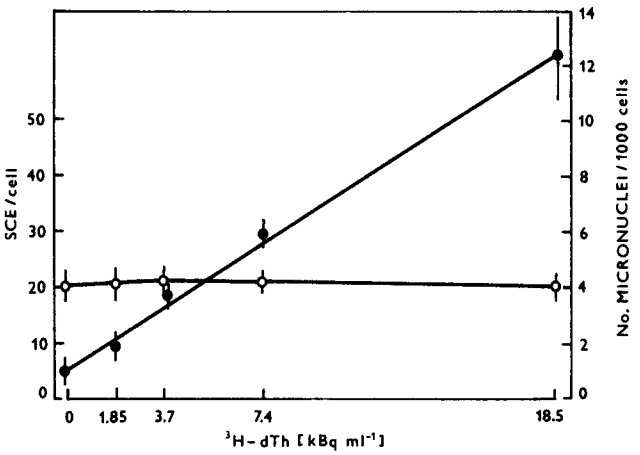

Fig. 1. The effects of a 24 h treatment with ^{3}H-dTh on the induction of SCEs (○) and micronuclei (●) in *Vicia faba* root tip cells.

The effects of pretreatment with ^{3}H-dTh on radiation-induced SCEs and micronuclei

The aim of the following experiments was to evaluate the consequences of low-level chronic beta-irradiation from ^{3}H-dTh with respect to the formation of SCEs and micronuclei in subsequently gamma-irradiated cells. For SCE induction, the roots were, after ^{3}H-dTh pretreatment, irradiated with a dose of 1.5 Gy. The results showed that the pretreatment with ^{3}H-dTh did not affect the levels of SCEs induced by subsequent gamma-irradiation. As follows from Tab. 1, the frequencies of SCEs after combined exposure to ^{3}H-dTh and 1.5 Gy of gamma-rays did not differ significantly from the yield of SCEs induced by gamma-rays separately.

The pretreatment with ^{3}H-dTh influenced, however, the yields of micronuclei after subsequent gamma-irradiation (Fig. 2). When cells chronically exposed to low concentrations of ^{3}H-dTh (1.85–3.7 kBq ml^{-1}) were irradiated with 1.5 Gy of gamma-rays, the frequencies of micronuclei were less than the yield induced by gamma-rays separately. In the cells pretreated with high concentration of ^{3}H-dTh (18.5 kBq ml^{-1}), the yield of micronuclei was rather at the additivity level.

Table 1
The frequencies of SCEs per S chromosome (± standard error) after a 24 h pretreatment with ^{3}H-dTh and subsequent gamma-irradiation with a dose of 1.5 Gy in *Vicia faba* root tip cells.

Treatment	SCE/S chromosome	Statistical significance
None	1.42 ± 0.08	–
^{3}H-dTh (1.85 kBq ml^{-1})	1.44 ± 0.08	$t = 0.125 < t_{05}$
^{3}H-dTh (3.7 kBq ml^{-1})	1.58 ± 0.09	$t = 1.331 < t_{05}$
^{3}H-dTh (18.5 kBq ml^{-1})	1.34 ± 0.12	$t = 0.422 < t_{05}$
1.5 Gy	2.35 ± 0.08	–
^{3}H-dTh (1.85 kBq ml^{-1}) + 1.5 Gy	2.23 ± 0.17	$t = 0.628 < t_{05}$
^{3}H-dTh (3.7 kBq ml^{-1}) + 1.5 Gy	2.56 ± 0.14	$t = 1.378 < t_{05}$
^{3}H-dTh (18.5 kBq ml^{-1}) + 1.5 Gy	2.42 ± 0.13	$t = 0.496 < t_{05}$

DISCUSSION

Our results demonstrate that 24 h low-level chronic beta-irradiation from ^{3}H-dTh does not affect the SCE frequency observed after subsequent gamma-irradiation in *Vicia faba* root tip cells. Thus, an effect similar to adaptive response to ionizing radiation on the level of SCEs was not found in our experiments. These results disagree with those describing the induction of an adaptive effect detectable by SCEs after pretreatment with ^{3}H-dTh in Chinese hamster cells (Ikushima 1987). On the other hand, conflicting results with regard to the presence or absence of adaptation to SCE induction were reported from different experiments in animal cells (Schubert and Heindorff 1989).

One of the possible explanations of our findings could be low ability of ionizing radiation to induce of SCEs. Indeed, although beta-radiation emitted from incorporated tritium is efficient in producing chromosomal aberrations, this radiation is a poor inducer of SCEs (for review, see Nakai and Tada-Aki Hori 1983). Our experiments have demonstrated that low-level chronic beta-irradiation resulting in the formation of micronuclei does not influence the frequencies of SCEs. Moreover, as was recently reported (Kuglík *et al.* 1989), the ability of gamma-radiation to induce SCEs in *Vicia faba* root tip cells depends on BrdU-incorporation into the chromosomal DNA and the incidence of SCEs in the cells with native DNA was effectively raised by relatively high radiation doses (1–4 Gy). For these reasons it is possible that ^{3}H-dTh either triggered the repair of a part, or such types of gamma-ray-induced primary lesions in DNA cannot be manifested in the total yield of SCEs.

In *Vicia faba* cells, similar negative results were reported by Schubert and Heindorff (1989) when, low dose pretreatment with chemical mutagens (maleic hydrazide, mitomycin C, N-methyl-N-nitrosourea) or heat shock were tested with regard to their effect on SCE induction by high doses of the same mutagens. These authors suppose that the removal of DNA lesions before or after replication could be responsible for the conflicting results with respect to the presence or absence of adaptation on the level of SCEs.

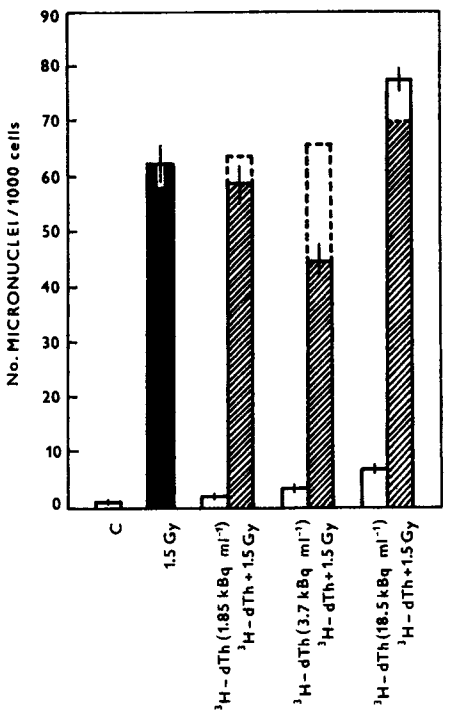

Fig. 2. The induction of micronuclei after a 24 h pretreatment with ^{3}H-dTh and subsequent gamma-irradiation with a dose of 1.5 Gy in *Vicia faba* root tip cells. Dotted lines show the yields expected on the basis of additive effects of ^{3}H-dTh and gamma-rays.

The results of our experiments proved that the pretreatment with ^{3}H-dTh decreased the level of chromosomal damage induced by subsequent gamma-irradiation when the frequencies of micronuclei were measured. This phenomenon is most likely due to beta radiation, because non-radioactive thymidine at the same concentrations did not affect the response of the cells (unpublished results). Due to the fact that micronuclei are mostly derived from acentric chromosomal fragments persisting without normal segregation in karyokinesis, these results are in agreement with those describing clastogenic adaptation in *Vicia faba* root tip cells after pretreatment with low doses of chemical mutagens or heat shock (Rieger *et al.* 1982, 1984, 1986).

The data obtained during the past year from experiments performed to characterise clastogenic adaptation suggest that there exist at least two differential inducible DNA repair systems in *Vicia faba* root tip cells: one, specifically acting on damage introduced into DNA by alkylating agents, and the other, probably specific to oxidative lesions in DNA (*cf.* Heindorff *et al.* 1987b).

Ionizing radiation is known to produce a variety of DNA lesions (for review, see Hutterman *et al.* 1978) and for these reasons very little is known about the molecular basis of the mechanisms underlying the adaptive response. Nevertheless, from the data available in the literature it is obvious that this phenomenon involves inducible chromosomal responses which can be apparently evoked when very low doses of both endogenous and external radiation are used. In human lymphocytes adaptive response to ionizing radiation at the chromosomal level could be induced by doses of 0.5–1 rad (Shadley and Wolff 1987). Furthermore, this response proved to be dependent on poly(ADP-ribosyl)ation and the amount of NAD in the medium (Wiencke *et al.* 1986, Wiencke 1987). In addition, higher resistance to radiation-induced chromosomal DNA damage and cross-resistance have been observed both in animal (Wolff *et al.* 1988, Vijayalaxmi and Burkhart 1989) and plant cells (Heindorff *et al.* 1987a) after pretreatment with bleomycin, S-independent radiomimetic agent inducing strand breaks in DNA (Povirk *et al.* 1977). For these reasons, namely strand breaks DNA-repair mechanism is suspected to be responsible for the low-doses-induced increase of the repair capacity observed in pre-irradiated cells.

REFERENCES

Cortés, F., Andersson, H. C.: Analysis of SCEs in *Vicia faba* by a simple fluorescent plus Giemsa technique. – Hereditas **107** : 7–13, 1987.

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.

Hutterman, J., Kohnlein, W., Teoule, R., Bertichamps, A. J. (eds.): Effects of Ionizing Radiation on DNA. – Springer-Verlag, Berlin 1978.

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

Kihlman, B. A., Andersson, H. C.: Root tips of *Vicia faba* for the study of the induction of chromosomal aberrations and sister chromatid exchanges. – In: Kilbey, B. J., Lecator, M., Nichols, W., Ramel, C. (ed.): Handbook of Mutagenicity Test Procedures. Pp. 531–554. Elsevier, Amsterdam 1984.

Kuglík, P., Šlotová, J., Karpfel, Z.: Effect of gamma radiation and postirradiational recovery on the formation of sister chromatid exchanges in meristematic cells of *Vicia faba*. – Biológia **44**: 617–622, 1989.

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.

Leenhouts, H. P., Broertjes, C., Sijsma, M. J., Chadwick, K. H.: Radiation stimulated repair in *Saintpaulia:* its cellular basis and effect on mutation frequency. – Environ. exp. Bot. **22** : 301–306, 1982.

Nakai, S., Hori, T.: Chromosome aberrations induced by incorporated tritium. – In: Ishihara, S., Sasaki, M. S. (ed.): Radiation-Induced Chromosome Damage in Man. Pp. 71–92. Alan R. Liss, New York 1983.

Olivieri, G., Bodycote, J., Wolff, S.: Adaptive response of human lymphocytes to low concentrations of radioactive thymidine. – Science **223** : 594–597, 1984.

Povirk, L. F., Wübker, W., Köhnlein, W., Hutchinson, F.: DNA-double strand breaks and alkali-labile bonds produced by bleomycin. – Nucl. Acids Res. **4** : 3573–3580, 1977.

Rieger, R., Michaelis, A., Nicoloff, H.: Inducible repair processes in plant root tip meristem? "Below-additivity effects" of unequally fractionated clastogen concentrations. - Biol. Zentralbl. **101** : 125–138, 1982.

Rieger, R., Michaelis, A., Nicoloff, H.: "Clastogenic adaptation" of the *Vicia faba* root-tip meristem as affected by various treatment parameters. – Mutat. Res. **104** : 99–102, 1984.

Rieger, R., Michaelis, A., Nicoloff, H.: Effects of stress factors on the clastogen response of *Vicia faba* root tip meristem – "clastogenic adaptation". – Biol. Zentralbl. **105** : 19–28, 1986.

Samson, L., Cairns, J.: A new pathway for DNA repair in *E. coli.* – Nature **267** : 281–283, 1977.

Samson, L., Schwartz, J. L.: Evidence for an adaptive DNA repair pathway in CHO and human skin fibroblast cell lines. – Nature **287** : 861–863.

Sankaranarayanan, K. A., V.Duyn, Loos, M. J., Natarajan, A. T.: Adaptive response of human lymphocytes to low-level radiation from radioisotopes or X-rays. – Mutat. Res. **211** : 7–12, 1989.

Shadley, J. D., Wolff, S.: Very low doses of X-rays can cause human lymphocytes to become less susceptible to ionizing radiation. – Mutagenesis **2** : 95–96, 1987.

Schubert, I., Heindorff, K.: Are SCE frequencies indicatives of adaptive response of plant cells? – Mutat. Res. **211** : 301–306, 1989.

Sybenga, J., Kleijer, G.: "Below-additivity" and "protective effects" of dose fractionation in *Crotolaria intermedia.* – Mutat. Res. **34** : 131–140, 1976.

Vijayalaxmi, Burkart, W.: Resistance and cross-resistance to chromosome damage in human blood lymphocytes adapted to bleomycin. – Mutat. Res. **211** : 1–5, 1989.

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

Wiencke, J. K.: Nicotinamide deficiency in human lymphocytes prevents the ^{3}H-thymidine-induced adaptive response for the repair of X-ray-induced chromosomal damage. – Exp. Cell Res. **171** : 518–523, 1987.

Wolff, S., Afzal, V., Wiencke, J. K., Olivieri, 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.