Mutation Research, 176 (1987) 205–209
Elsevier

MTR 04290

Micronucleus induction by low doses of X-rays in *Vicia faba* root tips

M. Rizzoni [a], E. Vitagliano [b], M.C. Marconi, A. Sottili and B. Gustavino [a]

[a] *Dipartimento di Biologia, Facoltà di Scienze Matematiche, Fisiche e Naturali, II Università di Roma, 'Tor Vergata'* and [b] *Centro di Genetica Evoluzionistica del C.N.R., c/o Dipartimento di Genetica e Biologia Molecolare, Facoltà di Scienze Matematiche, Fisiche e Naturali, I. Università di Roma, 'La Sapienza' (Italy)*

(Received 17 June 1986)
(Revision received 16 September 1986)
(Accepted 19 September 1986)

Key words: Micronucleus induction; X-Rays, low doses; Root tips; (*Vicia faba*).

Summary

Studies on the micronucleus test in *Vicia faba* root tips (VM test) were carried out in order to estimate the effects at low doses of X-rays (1, 2, 4, 8 and 12 R). The control value of micronucleus frequency is about 0.44/1000 cells. The dose where the micronucleus frequency is twice that of the control was estimated at 1.384 R. There was a linear kinetic dose response for the low-dose range studied here.

Mutagenesis tests based on plant systems generally show a good predictive value for mutagenic and carcinogenic effects on human beings and are solely adapted for environmental research "in situ" (for a recent review see Constantin and Owens, 1982). The cytogenetic tests with *Vicia faba* root tips are especially recommended for these purposes (for a recent review see Ma, 1982).

The possibility of inducing micronuclei by mutagens in *Vicia faba* root tips was discovered many years ago (Thoday, 1951; Read and Kihlman, 1956). Further research into this system brought to light the relationship between micronuclei and chromosome aberrations following irradiation with γ-rays and fast neutrons (Evans et al., 1959).

Recently this relationship was more deeply and systematically investigated in order to verify whether the micronucleus test in *Vicia faba* root tips (VM test) could be adopted as a warning signal capable of detecting mutagenic pollution in fresh water (Degrassi and Rizzoni, 1982). It was demonstrated that this test reveals a wide spectrum of mutagenic damage (both chromosomal aberrations and mitotic anomalies). A constant low control value was found as well as a good correlation between the frequencies of micronuclei and of both chromosome aberrations and mitotic anomalies (generating events). The frequency of the micronuclei is an indicator with a higher power of resolution when compared to the frequency of generating events.

In the research described here the response to a weak mutagenic action was studied in order to define the appropriate standard experimental procedures for the determination of the damage directly "in situ". With this aim in mind, X-rays were utilized as a simple mutagenic agent to minimize uncontrolled variables (pharmacokinetic,

Correspondence: Dr. Eleonora Vitagliano, Centro di Genetica Evoluzionistica CNR, c/o Dipartimento di Genetica e Biologia Molecolare, Facoltà di Scienze M.F.N., I. Università di Roma, P. le Aldo Moro (Italia).

TABLE 1

MICRONUCLEUS FREQUENCY ON 1000 CELLS IN ROOT TIPS OF *Vicia faba* IRRADIATED WITH X-RAYS AT LOW DOSES (1, 2, 4, 8, 12 R) AND FIXED 26 h AFTER TREATMENT

Dose	Number of tips scored	Number of tips showing the following micronucleus frequency on 1000 cells													Average micronucleus frequency ± standard error	Dunnett *t* value	*p*
		0	1	2	3	4	5	6	7	8	9	10	11	12			
Control	50	31	16	3	0	0	0	0	0	0	0	0	0	0	0.44 ± 0.09	–	–
1 R	50	21	19	8	2	0	0	0	0	0	0	0	0	0	0.82 ± 0.12	2.053	< 0.05
2 R	50	16	19	8	5	1	1	0	0	0	0	0	0	0	1.18 ± 0.17	3.999	< 0.01
4 R	25	6	8	4	5	0	2	0	0	0	0	0	0	0	1.64 ± 0.29	–	–
8 R	25	2	2	5	6	4	2	3	0	1	0	0	0	0	3.28 ± 0.39	–	–
12 R	25	1	0	3	3	5	4	2	3	1	1	1	0	1	5.08 ± 0.55	–	–

metabolism) and to enable the response of the VM test to be evaluated "in se", with low doses, too.

Materials and methods

The *Vicia faba* seeds, *minor* variety weibullus akerböna, which were kindly supplied by Dr. Palitti, were stored in a dry atmosphere at a temperature of +4°C until use. The Kihlman technique for germination was followed (Kihlman, 1977). At the moment of treatment the roots were 2–4 centimeters long. The tips were irradiated in water (180 kV, 6 mA, 3 mm Al) in a closed plastic petri dish, with the tips pointing towards the centre in order to minimize the distance variability between the roots and the source of X-rays (average distance was about 40 cm). For a check on the amount of doses a PTV Simplex dosimeter was used. The tips were irradiated with 1, 2, 4, 8, 12 R (15 R/min) and fixed 26 h after treatment using a 3 : 1 mixture of methanol and acetic acid. The Feulgen method was followed for staining. Slides were made by squashing in 45% acetic acid. During squashing the roots were cut off at about 1.5 mm starting off from the distal end. The permanent slides were prepared with Canada balsam. The fixing time corresponds to the time of higher frequency of the micronuclei for "low" doses (on the basis of results obtained with 30 R, see Degrassi and Rizzoni, 1982; see also Marshall and Bianchi, 1983) because it allows collection of micronuclei arising from chromosomal aberrations induced over a whole cell cycle. 50 tips were studied both for the control samples and for the 1 R and 2 R irradiated ones. 25 tips for 4 R, 8 R and 12 R irradiated samples were also studied.

The frequency of micronuclei was calculated on the basis of 1000 meristematic cells per tip.

The mitotic index on the same 1000 cells in the same tips was calculated too.

Results and discussion

Data are illustrated in Table 1 and in Fig. 1.

Mitotic index values did not show a remarkable variation as a function of X-rays dose, as could be expected at such low doses. Therefore a direct estimation of micronucleus frequency can be done without any correction (see Marshall and Bianchi, 1983).

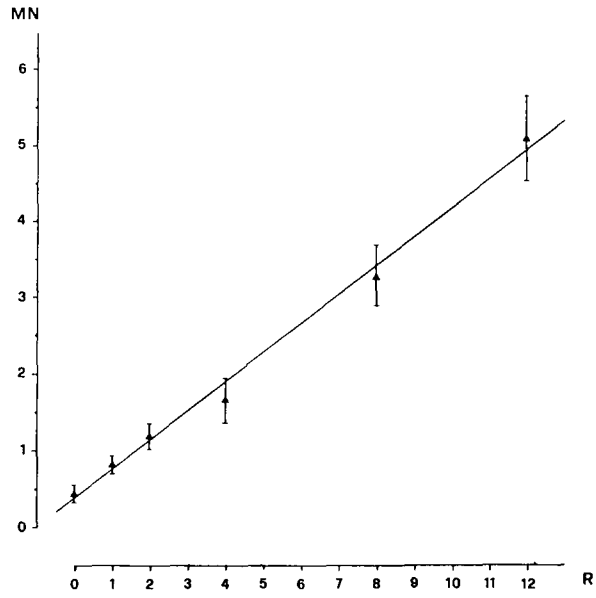

Fig. 1. Average frequency (± the standard error) of micronuclei (MN) in 1000 cells induced by low dose X-irradiation. The average micronucleus frequency was evaluated in 50 (control, 1 R, 2 R) and 25 (4 R, 8 R, 12 R) tips, 1000 cells per tip. Abscissa: X-rays dose (R). Ordinate: MN average frequency on 1000 cells.

A noticeable increase ($p < 0.01$) is registered in the frequency of micronuclei, using the Dunnett test, down to 2 R (see Table 1) when passing the micronuclei frequency per 1000 cells from 0.44 (control) to 0.82 (1 R) to 1.18 (2 R). There is a strong correlation between X-ray doses and micronuclei frequencies ($r = 0.995$).

Linear kinetics are shown in the range 0–12 R. When data are fitted by the method of least squares to $y = a + bx^n$, n has a value of 0.993. It was thus possible to calculate the dose in which the micronucleus frequency value is twice that of the control (1.384 R), through linear interpolation with the least squares method.

A more complex response is demonstrated (perhaps linear plus quadratic) if these results are compared to the results achieved by the same researchers on the same material with higher doses, like 30 and 60 R (Degrassi and Rizzoni, 1982). A similar result (linear + quadratic kinetics with prevalence of a linear component at low doses)

TABLE 2

COMPARISON BETWEEN DIFFERENT MICRONUCLEUS TEST SYSTEMS AS TO THE RESPONSE TO LOW-DOSES X-IRRADIATION

The values presented here were elaborated on the basis of the original data published in the literature cited. (MN, micronucleus frequency.)

Micronucleus test system	MN/cell control value	T.C.D. [a] (R)	MN/cell/R	References
Tradescantia pollen mother cells	0.01	4.17	0.0024	Ma, 1979
mouse polychromatic erythrocytes	0.032	4.64	0.0069	Jenssen and Ramel, 1978
human lymphocytes	0.005	20.00	0.00025	Countryman and Heddle, 1976
rat early spermatids	0.0025	19.23	0.00013	Lahdetie and Parvinen, 1981
Vicia faba root tips	0.00044	1.38	0.00038	present paper
Vicia faba root tips [b]	0.0013	7.65	0.00017	Marshall and Bianchi, 1983

[a] Doses where micronucleus frequencies are twice those of the control.
[b] Irradiated with γ-rays.

was obtained recently with the same system, by irradiating with 60 Co-γ-rays (Marshall and Bianchi, 1983).

Linear kinetics in the induction of micronuclei with X-rays were present in Tradescantia pollen mother cells (Ma, 1979), in polychromatic erythrocytes of mice (Jenssen and Ramel, 1978), and in precocious rat spermatids (Lähdetie and Parvinen, 1981), whilst a progression close to linear kinetics was shown in human lymphocytes (index 1.2: Countryman and Heddle, 1976).

Linear + quadratic kinetics for micronuclei are not at all surprising. The micronuclei actually derive, in these conditions, from acentric fragments which can be produced both by chromosomal aberrations with linear kinetics (simple deletions) and by ones showing quadratic kinetics (aberrations with asymmetric reunion).

A rough comparison can be performed with other test systems in which micronuclei were induced by X-irradiation. The micronucleus frequency per R per cell (MN/R/cell) was used in order to estimate the sensitivity of the tests, and the dose where the micronucleus frequency is twice that of the control (T.C.D.) was used to estimate its precision. These parameters were obtained on the basis of the linear interpolation of our data and of literature data in the range of doses giving a linear response (Table 2).

Acknowledgements

Many thanks are due to Prof. F. Palitti for supplying the seeds of *Vicia faba* and to Prof. L. Terrenato for his valuable contribution and help in statistical methodology.

References

Constantin, M.J., and E.T. Owens (1982) Introduction and perspectives of plant genetic and cytogenetic assays, A report of the U.S. Environmental Protection Agency GeneTox Program, Mutation Res., 99, 1–12.

Countryman, P.I., and J.A. Heddle (1976) The production of micronuclei from chromosome aberrations in irradiated

cultures of human lymphocytes, Mutation Res., 41, 321–332.

Degrassi, F., and M. Rizzoni (1982) Micronucleus test in *Vicia faba* root tips to detect mutagen damage in fresh-water pollution, Mutation Res., 97, 19–33.

Evans, H.J., G.J. Neary and F.S. Williamson (1959) The relative biological efficiency of single doses of fast neutrons and γ-rays on *Vicia faba* roots and the effect of oxygen, II. Chromosome damage: the production of micronuclei, Int. J. Radiat. Biol., 3, 216–229.

Jenssen, D., and C. Ramel (1978) Factors affecting the induction of micronuclei at low doses of X-rays, MMS and dimethylnitrosamine in mouse erythroblasts, Mutation Res., 58, 51–65.

Kihlman, B.A. (1977) Root tips of *Vicia faba* for the study of the induction of chromosomal aberrations, in: B.J. Kilbey, M. Legator, W. Nichols and C. Ramel (Eds.), Handbook for Mutagenicity Test Procedures, Elsevier, Amsterdam, pp. 389–400.

Lähdetie, J., and M. Parvinen (1981) Meiotic micronuclei induced by X-rays in early spermatids of the rat, Mutation Res., 81, 103–115.

Ma, T.H. (1979) Micronuclei induced by X-rays and chemical mutagens in meiotic pollen mother cells of Tradescantia, A promising mutagen test system, Mutation Res., 64, 307–313.

Ma, T.H. (1982) Vicia cytogenetic tests for environmental mutagens, A report of the U.S. Environmental Protection Agency Gene-Tox Program, Mutation Res., 99, 257–271.

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

Read, J., and B.A. Kihlman (1956) Comparison of the effects of 8-ethoxycaffeine and X-rays on the cytology and growth of roots of *Vicia faba*, Hereditas, 42, 487–507.

Thoday, J.M. (1951) The effect of ionizing radiations on the broad bean root, X. Chromosome breakage and the lethality of ionizing radiations to the root meristem, Br. J. Radiol., 24, 572–576; 622–628.