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BRIEF COMMUNICATION

Sister chromatid exchanges induced by heavy metals in *Vicia faba*

S. GÓMEZ-ARROYO[a]*, J. CORTÉS-ESLAVA*, R.M. BEDOLLA-CANSINO*,
R. VILLALOBOS-PIETRINI**, M.E. CALDERÓN-SEGURA* and Y. RAMÍREZ-DELGADO*

Laboratorios de Citogenética y Mutagénesis** Ambientales, Centro de Ciencias de la Atmósfera, Universidad Nacional Autónoma de México, Ciudad Universitaria, Coyoacán 04510 D.F. México*

Abstract

The induction of sister chromatid exchanges (SCE) by chloride and nitrate salts of nickel, cobalt, cadmium and zinc were studied in meristematic root cells of *Vicia faba*. Salts of nickel, cobalt and cadmium significantly increased the frequency of SCE, whereas chloride and nitrate salts of zinc did not increase the frequency of SCE significantly above the spontaneous level. The reported data demonstrate that the induction of SCE in *Vicia faba* may represent a valuable bioindicator for detecting the cytogenetic damage of heavy metals.

Additional key words: cadmium, chloride and nitrate salts of heavy metals, cobalt, field beans, nickel, root meristematic cells, zinc.

Vicia faba is a sensitive and reliable system for detecting sister chromatid exchanges (SCE) induced by chemical agents (Gómez-Arroyo and Villalobos-Pietrini 1995). Heavy metals represent important chemical pollutants, especially in industrial regions. Due to the fact that *Vicia faba* has proved to be a very sensitive biomonitor of water contaminants both in laboratory and *in situ* treatments (Gómez-Arroyo *et al.* 1988, 1997, Grant *et al.* 1992, Villalobos-Pietrini *et al.* 1994, Gómez-Arroyo and Villalobos-Pietrini 1995), the present study is aimed to detect the SCE induction of chloride and nitrate salts of four heavy metals: nickel, cobalt, zinc and cadmium.

Vicia faba L. (var. *minor*) seeds were germinated between two cotton layers soaked with tap water. Primary roots reaching 2 - 3 cm were treated with 100 μM 5-bromo-2'-deoxyuridine (BrdU), 0.1 μM 5-fluorodeoxyuridine (FdU) and 5 μM uridine (Urd) (*Sigma Chemical Co.*, St. Louis, USA) for 20 h. Afterwards, they were treated for 3 h with chloride and nitrate salts of nickel, cobalt, zinc and cadmium (purchased from *J.T. Baker, S.A.*, Xalostoc, México) dissolved in distilled water. Control roots were treated with distilled water. Fresh solutions of BrdU, FdU and Urd were applied for a second replicative cycle (20 h). The treatments were carried out in the dark at 20 °C. Two experiments were ran for each concentration.

The root tips were cut and treated with colchicine (0.05 %) for 3 h and stained using the Feulgen differential technique described by Tempelaar *et al.* (1982), modified as follows: cuttings were fixed with glacial acetic acid for 1 h, then put in ethanol-acetic acid (3:1) for 2 d at -2 °C, and later in 70 % ethanol for 15 min and hydrolysed in 5M HCl for 80 min at 20 °C. Then, they were washed three times with distilled water and stained with the Schiff reagent (Feulgen staining) for 12 min in the dark. Cuttings were treated with pectinase 2 %, dissolved in 0.01 M citrate buffer, pH 4.7, for 15 min at 28 °C, followed by acetic acid 45 % for 10 min and were finally transferred to cold ethanol for 30 min.

The cell squash was prepared in 45 % acetic acid. Slides were made permanent by a dry-ice technique, dehydrated by two absolute butanol changes, and then mounted in Canada balsam. Slides were coded and SCE was scored in 25 metaphase cells for each concentration in each one of the replicate experiments.

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[a] Corresponding author; fax: (+525) 6160789, e-mail: slga@ccaunam.atmosfcu.unam.mx

SCE were analyzed statistically using analysis of variance (*ANOVA*) to determine if significant difference existed among treated and control groups. When a significant F value was found ($P < 0.05$), the Newman-Keuls multiple comparison test was used to identify groups showing evidence of significant differences at $P < 0.001$ when compared with controls.

Both nickel salts produced SCE in a dose-response manner. The nitrate salt was more effective than the chloride salt and thus more toxic (Table 1). The regression line slope of nickel nitrate (correlation coefficient, $r = 0.93$) was higher than of nickel chloride ($r = 0.85$). In *Vicia faba*, both salts also induced chromosomal aberrations (Gläss 1955, 1956). However, no significant increase of point mutations (Miyaki *et al.* 1979, Umeda and Nishimura 1979) and chromosomal aberrations (Paton and Allison 1972) in mammal cells *in vitro* were observed.

Table 1. Sister chromatid exchanges induced by nickel chloride and nickel nitrate in *Vicia faba*. Means ± SE, $n = 50$ metaphases in two experiments, * significant difference from the control at $P < 0.001$. At higher concentrations no metaphases were observed.

$NiCl_2$ [μM]		$Ni(NO_3)_2$ [μM]	
0	29.72 ± 0.73	0	29.72 ± 0.73
0.004	34.84 ± 0.72*	0.003	39.84 ± 0.73*
0.042	35.96 ± 0.80*	0.034	52.12 ± 0.31*
0.420	41.16 ± 0.95*	0.340	-

Both cobalt salts increased the SCE frequencies when higher concentrations were applied. Chloride was more effective than nitrate in inducing SCE as well as toxicity, as chloride doubled the basal frequency of SCE at 0.68 μM and the highest frequency was at 51.74 μM (3.4 times higher compared with control). The toxic effect started with 68.98 μM for $CoCl_2$and 51.54 μM for $Co(NO_3)_2$ (Table 2). The correlation coeficients were 0.89 for $CoCl_2$ and 0.91 for $Co(NO_3)_2$. In *Vicia faba* and *Allium cepa* $CoCl_2$, $Co(NO_3)_2$, and $CoSO_4$ produced nuclear alterations, decrease of cell division, stickiness of chromosomes, chromosomal aberrations and aneuploidy (Gläss 1955, 1956, Komczynski *et al.* 1963, Herich 1965). In mammalian cells cobalt salts induced, *i.e.*, mutations in HPRT locus in V79 cells (Miyaki *et al.* 1979, Hartwig *et al.* 1990, 1991), micronuclei in human lymphocytes (Capomazza and Botta 1991), in mouse bone marrow cells (Suzuki *et al.* 1993), SCE in macrophage cell line P388D and human lymphocytes (Andersen 1983) and SCE in Chinese hamster V79 cells (Hartwig *et al.* 1991).

Table 2. Sister chromatid exchanges induced by cobalt chloride and cobalt nitrate in *Vicia faba*. Means ± SE, $n = 50$ metaphases in two experiments, * significant difference from the control at $P < 0.001$. At higher concentrations no metaphases were observed.

$CoCl_2$ [μM]		$Co(NO_3)_2$ [μM]	
0	29.58 ± 0.54	0	29.58 ± 0.54
0.68	60.62 ± 1.04*	0.34	48.20 ± 1.20*
6.89	71.70 ± 1.78*.	3.43	59.36 ± 1.30*
34.49	95.80 ± 0.98*	17.18	80.96 ± 1.24*
51.74	105.80 ± 0.54*	25.77	86.54 ± 0.74*
68.98	-	34.36	89.80 ± 0.65*

Both zinc salts did not induce SCE in *Vicia faba*. The r values were 0.50 for $ZnCl_2$ and 0.17 for $Zn(NO_3)_2$. These salts were less toxic than those of nickel and cobalt, because they did not inhibit the cell division up to 36.60 μM for chloride and 168.07 μM for nitrate. Zinc salts produced chromosomal alterations in *Allium cepa* (Mitra 1984) and in *Vicia faba* (Gläss 1956). The metal has evoked mutagenic activity in mammalian cells *in vitro* (Amacher and Paillet 1980, Gasiorek and Bauchinger 1981) as well as in *in vivo* systems (Giri *et al.* 1988).

Table 3. Sister chromatid exchanges induced by zinc chloride and zinc nitrate in *Vicia faba*. Means ± SE, $n = 50$ metaphases in two experiments, the differences were not significant from the control at $P < 0.001$. At higher concentrations no metaphases were observed.

$ZnCl_2$ [μM]		$Zn(NO_3)_2$ [μM]	
0	29.30 ± 0.48	0	29.58 ± 0.48
0.73	32.26 ± 0.81	0.33	32.94 ± 0.93
7.32	29.88 ± 0.94	3.36	30.58 ± 0.66
36.60	29.66 ± 0.81	16.80	30.18 ± 0.74
73.20	31.40 ± 0.60	33.61	29.68 ± 0.41
146.41	30.64 ± 0.52	67.22	29.54 ± 0.50
219.61	31.00 ± 0.50	100.84	29.58 ± 0.58
292.82	32.28 ± 0.65	134.45	32.92 ± 0.87

When *Vicia faba* root tips were exposed to cadmium nitrate, the SCE frequencies were significantly higher than the control values and at the highest concentration (0.324 μM), the frequency was doubled (Table 4). The r values were 0.91 for $CdCl_2$ and 0.90 for $Cd(NO_3)_2$. Cadmium chloride produced chromosomal aberrations as well as spindle disturbances in *Allium cepa, Beta vulgaris* (Rosen 1954), *Hordeum vulgare, Nigella damascena* (Degraeve 1971), *Crepis capillaris* (Ruposhev and Garina 1976), *Allium sativum* (Mukherjee *et al.* 1984), and *Eichhornia crassipes* (Rosas *et al.* 1984). In mammalian cells cadmium sulfide induced chromatid and isochromatid breakage and translocations in human lymphocytes in culture (Shiraishi *et al.* 1972), whereas in

other cell lines, the effects were observed only at toxic doses (Mukherjee *et al.* 1984). Although $CdCl_2$ was not clastogenic in mammalian systems *in vitro* (Sharma and Talukder 1987), cadmium acetate was a weak clastogen (Gasiorek and Bauchinger 1981).

Table 4. Sister chromatid exchanges induced by cadmium chloride and cadmium nitrate in *Vicia faba*. Means ± SE, $n = 50$ metaphases in two experiments, * significant difference from the control at $P < 0.001$. At higher concentrations no metaphases were observed.

$CdCl_2$ [μM]		$Cd(NO_3)_2$ [μM]	
0	30.00 ± 0.89	0	32.04 ± 0.75
0.004	31.02 ± 0.92	0.003	32.98 ± 0.90
0.043	35.76 ± 1.13*	0.032	53.65 ± 2.04*
0.437	36.41 ± 0.88*	0.324	73.02 ± 1.85*
2.189	36.81 ± 1.55*	1.620	-

When the regression lines of the salts, expressing the SCE inducing activity in relation to the applied concentration, were compared, the rank order was: nickel nitrate > cadmium nitrate > nickel chloride > cobalt chloride > cobalt nitrate > cadmium chloride > zinc nitrate = zinc chloride. The mean number of SCE per metaphase in control *Vicia faba* roots for chromosomes TB-BB (from two replicative cycles in presence of BrdU) was 29.30 to 29.76 (Tables 1 to 4), which agreed well with 29 SCE per metaphase obtained by Kihlman and Andersson (1984) and in other studies (Gómez-Arroyo *et al.* 1988, 1997, Gómez-Arroyo and Villalobos-Pietrini 1995, Calderón-Segura *et al.* 1999).

The reported data demonstrate that the induction of SCE in *Vicia faba* represents a valuable bioindicator for detecting the cytogenetic damage of heavy metals.

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