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Induction of sister chromatid exchanges by heavy metal salts in root meristem cells of *Allium cepa* L.

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Abstract

Four heavy metal salts, nickel sulphate, mercuric chloride, cadmium sulphate and zinc sulphate, were tested for induction of sister chromatid exchange (SCE) in root meristem cells of *Allium cepa*. A simple modified Feulgen staining procedure was employed for SCE-analysis. Maleic hydrazide and paraquat were included for comparison. An evaluation of genotoxicity of the above test chemicals made on the basis of SCE-assay was found positive for all the test chemicals with exception of zinc sulphate which gave a weak positive result.

Additional key words: cadmium, chemical mutagens, dose-response, maleic hydrazide, mercury, nickel, onion, paraquat, zinc.

Introduction

Sister chromatid exchanges (SCE) assay has been proved to be one of the most sensitive short-term genotoxicity assay owing to its ability to detect genotoxicants at very low concentrations (Tucker *et al.* 1993). Although the exact mechanism(s) of induction of SCE is still a subject of discussion (Cortes *et al.* 1994), SCE are widely believed to represent the interchange of DNA replication products at apparently homologous loci, and involve DNA breakage and reunion (Latt *et al.* 1981).

The mammalian *in vivo* or *in vitro* SCE assays mostly employ the fluorescent-plus-giemsa (FPG) technique (Perry and Wolf 1974). In plant cells SCE have been demonstrated by application of the FPG technique (for review see Schvartzman

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Abbreviations: BrdUrd - 5-bromodeoxyuridine; dT - deoxythymidine; FdUrd - 5-fluorodeoxyuridine; FPG - fluorescent-plus-giemsa; MH - maleic hydrazide; PQ - paraquat; SCE - sister chromatid exchanges; Urd - uridine.

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1987). SCE in plants, however, are less frequently studied due to certain inherent problems associated with plant cells (Gerster and Grant 1989). Consequently, there are not many chemicals that have been tested for induction of SCE in plant cells.

Nevertheless such information is highly essential not only for the purpose of comparison with SCE from mammalian assays but also for a better understanding of the overall mechanism of SCE-induction. Furthermore, the simplicity of plant assays, the low relative cost, their versatility and the minimal laboratory facilities required for environmental monitoring, so much so they have been the assays of choice for genetic toxicological testing in the developing countries throughout the world (De Serres 1992, Grant 1994).

In the present paper four metal salts, namely nickel sulphate, mercuric chloride, cadmium sulphate and zinc sulphate are tested for induction of SCE in root meristem cells of *Allium cepa* L. A simple Feulgen staining procedure as a substitute to the FPG-technique is applied for differential staining of SCE. Two chemical mutagens, namely maleic hydrazide (MH) and paraquat (PQ) which are believed to exert genotoxicity *via* oxidative mechanisms (Panda *et al.* 1995, Reddy *et al.* 1995) are included for comparison.

Materials and methods

Plants: The growing root meristems of *Allium cepa* L. (2n = 16) were used as the assay. Healthy bulbs of *A. cepa* were allowed to germinate by placing five bulb per cylindrical plastic receptacles containing 250 cm^3 of tap water at 24 ± 1 °C in dark. Air was bubbled through continuously at a rate of 10 - 20 cm^3 min^{-1} and the tap water was renewed daily.

Feulgen staining procedure for SCE assay. The Feulgen staining procedure (Gerster and Grant 1989) was used with some modification as follows. The growing root meristems, 2 - 3 cm long, were treated with 100 μM BrdUrd, 0.1 μM FdUrd and 5 μM Urd (*Sigma*, New Delhi, India) for 20 h (approximately one cell cycle) followed by a second round of treatment for 20 h with 100 μM dT (*Loba-Chemie*, Bombay, India) and 5 μM Urd. The treatments were terminated by washing the roots with running tap water and then treated with 0.05 % colchicine (*Loba-Chemie*, Bombay, India) for 2.5 h. All the treatments were made in plastic containers containing 250 cm^3 of experimental solutions and were under continuously bubbling. Roots were washed, excised and fixed in acetic acid:methanol (1:3) for 7 h and preserved in 70 % methanol at 4 °C. The excised root meristems were hydrolysed in 5 M HCl at 28 °C for 78 - 82 min and stained in Feulgen reagent for at least 3 h. The duration of fixation as well as hydrolysis standardized as above, are crucial for differential staining of sister chromatids. The stained root meristems were washed in distilled water and squashed in a drop of aceto-orcein 0.025 % and tapped for metaphase chromosome separation under coverslip. The coverslips were sealed with nail polish. The SCE were scored at 400-fold magnification. The temporary slides may be stored at 4 °C for about a week without affecting the quality of the stain. The

advantages with the present Feulgen techique over the FPG technique (Kihlman and Kronborg 1975, Cortes and Anderson 1987) are that a number of procedural steps such as *1)* treatment of roots with pectinase to facilitate maceration, *2)* removal of coverslip from the slide after squashing of the root, *3)* treatment with RNA-ase, *4)* treatment with the fluorochrome, *33258 Hoechst* and *5)* UV-irradiation are avoided in the Feulgen staining procedure, thereby saving both cost as well as time.

Exposure schedule for test chemicals and analysis of SCE: Following the first round of treatments with BrdUrd, FdUrd and Urd for 20 h, the growing root meristems were exposed to the test chemicals: MH (*British Drug House*, Poole, England), PQ (*Sigma*, New Delhi, India), nickel sulphate ($NiSO_4 . 7H_2O$; *Merck*, Bombay, India) mercuric chloride ($HgCl_2$; *BDH*, Bombay, India), cadmium sulphate ($3\ CdSO_4 . 8H_2O$; *Loba-Chemie*, Bombay, India) zinc sulphate ($ZnSO_4 . 7H_2O$; *BDH*; Bombay, India) at the desired concentrations for 1 h in presence of dT and Urd. Stock solutions of the test chemicals were made fresh and diluted with tap water to prepare experimental solutions of the concentrations desired. The pH of the tap water was 7. After a brief wash, the roots were subjected to the second round of treatments with dT and Urd for another 19 h. Subsequently the roots were treated with colchicine, washed, excised and fixed for analysis of SCE following the Feulgen staining procedure as described earlier. Parallel tap water controls were included and handled alike for each set of treatments.

At least 30 metaphase spreads from 5 or more root meristems for each concentration were analysed. From each metaphase spread at least 10 chromosomes were examined for SCE-analysis. Before cytological analysis the glass vials containing the roots were already coded. Increase in the frequency of SCE was tested statistically using one tailed *t*-test. Dose-response obtained for each of the test chemicals were subjected to regression analysis.

Results

All the test chemicals were tested at 3 or more concentrations in order to find if the increase in the induction of SCE (Table 1) follow any dose-response relationship. The bases for selecting the chemical concentrations were: *a)* at the concentrations tested the chemicals did not inhibit cell division as indicated by the mitotic indices (per cent of mitoses) that ranged between 6 % and 10 % (data not presented) so that a good number of scorable metaphases were available for SCE analysis, *b)* at concentrations below the range of concentrations presently tested the test chemicals apparently had no effect on SCE frequencies as compared to that of corresponding controls, and *c)* at concentrations above the range, the frequencies of SCE induced were either too high to be scored or had inhibitory effect on cell division so that enough metaphases were not available for SCE analysis.

Table 1. Dose-response induction of SCE in root meristem cells of *Allium cepa* by maleic hydrazide, paraquat, nickel sulphate, mercuric chloride, cadmium sulphate and zinc sulphate.

Test chemicals	Concentration [μM]	Metaphases analysed [number of roots]	Number of chromosomes analysed	Total number of SCE	Mean SCE per chromosome ± SE
Control	0	51 (8)	755	1228	2.0 ± 0.05
MH	0.1	40 (9)	598	1379	2.2 ± 0.02**
	1.0	40 (8)	574	1882	3.4 ± 0.01***
	10.0	38 (9)	548	3306	6.1 ± 0.02***
PQ	0.1	54 (9)	774	1962	2.1 ± 0.005*
	1.0	60 (10)	853	2742	3.1 ± 0.01***
	10.0	59 (9)	862	3836	4.5 ± 0.01***
Control	0	34 (5)	355	673	2.1 ± 0.02
$NiSO_4$	10.0	33 (5)	398	871	2.3 ± 0.01***
	100.0	39 (7)	384	1273	4.0 ± 0.01***
	1000.0	33 (6)	342	2345	5.4 ± 0.01***
Control	0	41 (8)	563	1171	2.1 ± 0.004
$HgCl_2$	0.4	39 (8)	527	1078	2.2 ± 0.01***
	4.0	40 (9)	538	1377	2.6 ± 0.01***
	40.0	41 (8)	453	2050	4.4 ± 0.02***
$CdSO_4$	0.04	40 (8)	563	1147	2.1 ± 0.005
	0.4	48 (8)	497	1339	2.8 ± 0.01***
	4.0	50 (7)	519	2016	3.8 ± 0.025***
	40.0	56 (8)	505	2196	4.4 ± 0.03***
$ZnSO_4$	10.0	40 (6)	550	1274	2.3 ± 0.01***
	100.0	40 (6)	556	1187	2.1 ± 0.004
	1000.0	40 (8)	535	1385	2.6 ± 0.01***

Significant at $P \geq 0.05$ (*), $P \geq 0.01$ (**) and $P \geq 0.001$(***) determined by one tailed *t*-test for comparison with control

The average SCE per chromosome was calculated to be 2.06 ± 0.002 ($n = 126$, CV < 1 %) when the values from all the controls were pooled together. In the present study, all of the test chemicals induced significant increase ($P \leq 0.001$) in the SCE frequencies at two of three concentrations. The increase of SCE-frequencies for MH ($r = 0.97$, $P \leq 0.05$) and $HgCl_2$ ($r = 0.99$, $P \leq 0.001$) followed a linear dose-response relationship. For $CdSO_4$ the SCE frequencies increased non-linearly with increase of dose ($r = 0.89$, $P \leq 0.05$). The increase in the frequencies of SCE induced by PQ ($r = 0.93$) and $NiSO_4$ ($r = 0.88$) apparently was dose dependent which however was not significant following the regression analysis. Although significant increase in the SCE frequencies was observed for $ZnSO_4$ at the lowest and the highest concentrations, a clear dose-response relationship was lacking. Furthermore, with exception of $ZnSO_4$ a doubling of SCE frequency above the corresponding controls was noted for the highest concentrations of all the chemicals.

Discussion

MH, a S-dependent clastogen, has been shown to be an inducer of SCE in plant cells (Cortes *et al.* 1987). In various mammalian cells *in vitro*, however, MH is known to induce SCE at significant levels only in higher concentrations between the range 100 and 8000 μM (Speit 1983). PQ which is known to be genotoxic through generation of superoxide radicals (Reddy *et al.* 1995) has been reported to induce SCE at significant levels in Chinese hamster lung Don cell line fibroblasts (Nicotera *et al.* 1985). The concentrations that doubled the SCE frequencies in the above mammalian cells *in vitro* over that of the respective controls for MH and PQ were 4000 and 500 μM, respectively. Compared to the above, the increase in the frequencies of SCE induced by MH and PQ were not only significant ($P \leq 0.05$ - 0.001) but also they both doubled the SCE frequencies at a far less concentration *i.e.*, 10 μM, the highest concentration tested (Table 1). The present findings with respect to MH is in agreement with a recent report on SCE induction by MH in root tip cells of *Vicia faba* (Veselská *et al.* 1995).

Of the metal salts presently tested, $NiSO_4$ at concentrations 5 - 50 μM has been reported to induce SCE at significant levels ($P \leq 0.05$) in mammalian cells *in vitro* (Ohno *et al.* 1982, Sahu *et al.* 1989). In the above studies doubling of SCE-frequency by $NiSO_4$ over that of control, however, was not shown. In the present study $NiSO_4$ induced significant increase of SCE ($P \leq 0.001$) at all the concentrations tested and further at 1000 μM the SCE-frequency was doubled over that of control.

Morimoto *et al.* (1982) reported that $HgCl_2$ at concentrations 0.01 - 50 μM induced SCE at significant levels ($P \leq 0.05$ or 0.01) in human lymphocytes *in vitro* that followed a dose-response but failed to double the SCE-frequency. $HgCl_2$, in the present study, induced SCE in significant frequencies over the control that followed not only a linear dose-response ($r = 0.99$, $P \leq 0.01$) but doubled the SCE-frequency at 40 μM, being the highest concentration tested.

Whereas information regarding the effects of $CdSO_4$ on SCE particularly is lacking, SCE-test results available for other salts of cadmium have been negative (Ohno *et al.* 1982). In the present study $CdSO_4$ increases SCE frequency at significant levels ($P \leq 0.001$) and the doubling of SCE-frequency was noted at 40 μM. The dose-response increases for $CdSO_4$, however, was non-linear ($r = 0.89$, $P \leq 0.05$).

The dose-response for $ZnSO_4$ was again not clear, although the frequencies of SCE induced were significant at 10 and 1000 μM (Table 1).

The information on induction of SCE by metal salts available for comparison thus have been from mammalian assays only. The foregone comparative account nevertheless suggested the present *Allium* SCE assay to be equally or even more sensitive than the mammalian SCE assays and therefore may routinely be employed in genetic toxicological testing of environmental chemicals.

In an attempt to evaluate the genotoxicity of chemicals on the basis of their ability to induce SCE in mammalian cells *in vitro* and *in vivo* a few criteria have been considered in order to discriminate negative, weak positive and clear positive

responses (Tucker *et al.* 1993). Based on the above criteria MH, PQ, $NiSO_4$, which have demonstrated both doubling of the frequency over the appropriate controls and a 3-point monotonic increase at significant levels ($P \geq 0.001$) as observed in the present study, may be considered as clear positive. $ZnSO_4$ which induced statistical significant increase ($P \geq 0.001$) at two out of the three considerations tested (a 3 point monotonic dose-response was absent) but failed to double the SCE-frequency is considered weak positive.

It may further be noted that almost all of the presently tested heavy metals induced DNA-strand breaks or DNA-damage through generation of active oxygen species (Kawanishi *et al.* 1994). Since active oxygen species have been implicated at the origin of SCE (Emerit *et al.* 1982), it seems likely that the present metal-induced SCE involved active oxygen species, warranting further research.

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