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Genotoxicity Testing of Some Metals in the *Drosophila* Wing Somatic Mutation and Recombination Test

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Heavy metals are important environmental pollutants. Metals in soil and water may enter the food chain. Food sources are being considered probably the largest source of exposure, second being air pollution. Further potential sources to which human are exposed, include consumer products and industrial wastes (Goyer 1991).

Many organ systems are effected by heavy metals which target specific biochemical processes and/or membranes of cells and organelles (Goyer 1991; Long 1997; Yan et al. 1997). Various organic and inorganic salts of heavy metals have been subjected to a variety of genotoxicity tests, but no clear conclusion can be driven from those reports (Codina et al. 2000; De Flora et al. 1990; De Flora et al. 1994; Hartwing 1998; Mayer et al. 1998; Ogawa et al. 1994; Sharma et al. 1988; Uysal and Bahçeci 1997; Winder and Bonin 1993). Direct genotoxic effects of some metals such as nickel, chromium, cobalt and arsenic are rather weak and/or restricted to relatively high concentrations (Hartwing 1998). Sharma et al. (1988) reported that lead acetate and lead nitrate failed to cause any mutation in *Salmonella* but lead chromate was an effective mutagenic agent in this organism. The salts of these metals caused a significant increase in the frequency of aberration in the polytene chromosomes of *Anopheles stephensi* and *Drosophila melanogaster* (Winder and Bonin 1993; Uysal and Bahçeci 1997). Furthermore, in *D. melanogaster* $MnCl_2$, $MoCl_3$, $NiCl_2$ and $ZnCl_2$ shown to have mutagenic effects, no such effect was reported for $CrCl_3$, $FeCl_2$ and $FeCl_3$ (Ogawa et al. 1994). The genotoxic effect of mercury, chromium, copper, nickel and zinc was determined by different microbial assays (Codina et al. 2000). In this study, some metals have been subjected to the genotoxicity test, using somatic mutation and recombination test (SMART) in *D. melanogaster*. SMART test relies on wing spots of *D. melanogaster* and is a rapid, inexpensive *in vivo* assay, which detects genotoxic agent using somatic cells of a higher eukaryote. In this test, both somatic mutation and mitotic recombination are screened. The test itself is quite sensitive to a wide variety of both direct-acting mutagens and the ones that require bioactivation (Graf et al. 1994; Graf et al. 1998; Yeşilada 1999; Yeşilada et al. 1999). As mentioned above there are some studies on genotoxic effects of heavy metals, but studies, which use the SMART test to detect the genotoxic activity of metals, are limited.

MATERIALS AND METHODS

Metal nitrate compounds used here were; $AgNO_3$, $Ba(NO_3)_2$, $Co(NO_3)_2$. $6H_2O$, $Cr(NO_3)_2$. $9H_2O$, $Fe(NO_3)_3$. $9H_2O$, $Hg(NO_3)_2$. H_2O, $Mn(NO_3)_2$. $4H_2O$, $Ni(NO_3)_2$. $6H_2O$, $Pb(NO_3)_2$ and $Zn(NO_3)_2$. $6H_2O$. The test concentration of these which itself served compounds were made in ddH_2O which itself served as the negative control. A 20 m*M* aqueous solution of ethyl methanesulfonate (EMS) used as a positive control. Metal nitrates and EMS were purchased from Merck (Durmstadt, Germany) and were reagent grade.

Two stocks of *D. melanogaster* were used. Virgin females from the strain flair (*flr*^{3}*/In(3LR)TM3, ri pp sep bx*^{34e}*e*^{s} *Ser*) were mated to males from the strain multiple wing hairs (*mwh*). *mwh* and *flr*^{3} recessive wing mutations are both located on the left arm of chromosome 3. Detailed information on the genetic markers of these stock organisms was given by Lindsley and Zimm (1992). The strains were obtained from the laboratory of Ulrich Graf, Swiss Federal Institute of Technology (ETH), University of Zurich. All experiments were conducted at 25±1° C and 60 % relative humidity on cornmeal-agar medium (Bozcuk 1978).

Eggs from crosses between the two strains mentioned above were collected during 8 hr and the larvae were floated off the food in 17% NaCl 72±4 hr after. Groups of 25 larvae were transferred to individual glass vials containing 2 mL of the food with 500 µL of the test solution on the surface. The larvae developed in this medium until pupation. The reliability of this method of exposure was verified by Marec and Socha (1987).

The adult flies eclosing from the treatment vials were collected on days 10-12 after egg laying. The number of eclosions was also counted and the survival rate was calculated. From the eclosed adult flies only trans-heterozygous (*mwh* +/+ *flr*^{3}) were collected and stored in 70% ethanol. Their wings were mounted in Faure's solution (gum arabic 30 g, glycerol 20 ml, chloral hydrate 50 g and water 50 mL) and inspected under 500X magnification to determine the spot size and their types. The observed wing hair spots were classified as small single spots, large single spots or twin spots according to the method of Graf et al. (1984). One or two *mwh* or *flr* mutant cells were scored as small single spots, three or more *mwh* or *flr* mutant cells as large single spots and neighboring *mwh* and *flr* mutant cells as twin spots. These 3 types of spots were evaluated separately. For the frequencies of spots per wing, a multiple-decision procedure was used to decide whether a result is positive, weakly positive, inconclusive, or negative (Frei and Würgler 1988). The wing spot data of treated and control series were compared by conditional binominal test. Each statistical test was performed at the 5% significance level. In these study, the frequency of clone formation per 10^5 cells was determined, based on the number of wings analyzed, the number of *mwh* clones recorded (i.e., *mwh* single spots and the *mwh* parts of twin spots), and the number of cells scored in each wing (approx. 24 400, a standard number for analyzing induced somatic spots) (Frei et al. 1992; Graf 1995).

RESULTS AND DISCUSSION

The larval survival ratio for all of groups and the results of SMART are summarized in Table 1. All concentrations of metals and EMS used in this study caused a decrease in larval survival. Heavy metals are well known for their effects in reducing the growth rate, viability and the protein synthesis (Salvado et al. 1997). The treatment at concentrations higher than 0.5 m*M* for $AgNO_3$ and 1m*M* for $Hg(N0_3)_2$ were determined to be lethal for larvae. Higher concentrations of the other metal nitrates used, caused a lower number of survivals, a reason for not being able to obtain enough amount of wings for SMART test.

The data obtained from the *Drosophila* wing spot test showed that most of the spontaneously occurring spots on the wings of control were the small single spots. The large single spots and twin spots were rare. In addition, all single spots showed the *mwh* phenotype. EMS, which was used as a positive control, induced all kinds of spots. These data documented the strong mutagenic and recombinogenic activity of EMS as reported by others (Graf et al. 1984; Marec and Socha 1987).

Ten m*M* of $Co(N0_3)_2$, and 0.5 m*M* of $AgNO_3$ increased the frequency of all types of spots. The lower concentration of $Co(NO_3)_2$ (1m*M*) had a positive result for the small single and the large single spots. But the results were inconclusive for the twin spots. A high concentration (10m*M*) for both $Ba(N0_3)_2$ and $Mn(NO_3)_2$ gave positive results for the small single spots, but results from the low concentrations (1m*M*) were inconclusive for all type of spots. $Ni(NO_3)_2$ at 10m*M* also produced positive results for the small single spots and the large single spots. The frequency of twin spots was also significantly increased with 1m*M* $Ni(NO_3)_2$ treatment. $Pb(N0_3)_2$ showed positive results for the small single spots and the large single spots types. At 1m*M* concentration, $Hg(N0_3)_2$ gave inconclusive results only for twin spots but gave positive results for the other spot types. The studied concentrations of $Cr(N0_3)_2$, $Fe(N0_3)_2$, and $Zn(N0_3)_2$ showed inconclusive results for all spot types.

Another way of presenting and evaluating the wing spot test data is to calculate the clone-formation frequency per 10^5 cells (Graf et al. 1994). These researchers reported that a frequency higher than 2.0 is indicative of genotoxic activity of a particular treatment. In the present study, while the clone formation frequency for the negative control was 0.1, it was 40.5 for the EMS positive control. This frequency was 4.0 for 10 m*M* concentration of $Co(N0_3)_2$ and it was 2.4 for 0.5 m*M* concentration of $AgNO_3$. For the other metal nitrate compounds, this value was between 0.2 and 0.6 (Table 1).

Some metals have been tested for genotoxic potential in a range of mutagenicity assays. In such studies it was reported that some metals induced DNA damage, including DNA strand breaks, DNA-protein crosslinks, chromosomal aberrations and sister chromatid exchanges (De Flora et al. 1990; Goyer 1991; Mayer et al. 1998). Small single spots and large single spots in SMART test are assumed to be due to gene mutations, chromosomal deletion, non-disjunction, or mitotic

Table 1. Summary of results obtained in the *Drosophila* wing somatic mutation and recombination test with some metals

Concn. (m*M*)		Survival Ratio (%)	Wings (no.)	Spots/ Wing [a] and Statistical Diagnosis [b] Small Single Spots m= 2.0	Large Single Spots m=5.0	Twin Spots m= 5.0	Total Spots m= 2.0	Spots with *mwh* clone	c
Control	(0)	92.06	300	0.03(8)	0.01(2)	0.00(0)	0.03(10)	10	0.1
EMS	(20)	74.29	27	5.67(153)+	3.48(94)+	0.89(24)+	10.04(271)+	267	40.5
$AgNO_3$	(0.5)	55.56	110	0.14(45)+	0.07(15)+	0.04(6)+	0.60(66)+	64	2.4
$Ba(NO_3)_2$	(1)	86.19	133	0.04(5)i	0.02(2)i	0.00(0)i	0.05(7)i	7	0.2
	(10)	61.33	102	0.08(8)+	0.03(3)i	0.01(1)i	0.12(12)+	12	0.5
$Co(NO_3)_2$	(1)	79.17	110	0.14(15)+	0.08(9)+	0.01(1)i	0.23(25)+	25	0.9
	(10)	16.41	49	0.74(36)+	0.16(8)+	0.08(4)+	0.98(48)+	48	4.0
$Cr(NO_3)_3$	(1)	77.14	112	0.03(3)i	0.02(2)i	0.00(0)i	0.05(5)i	5	0.2
	(10)	70.40	128	0.04(5)i	0.02(3)i	0.00(0)i	0.06(8)i	7	0.2
$Fe(NO_3)_3$	(1)	74.67	118	0.03(3)i	0.02(1)i	0.02(2)i	0.05(6)i	5	0.2
	(10)	64.76	175	0.03(5)i	0.01(2)i	0.01(1)i	0.05(8)i	8	0.2
$Hg(NO_3)_2$	(1)	26.66	59	0.17(5)+	0.10(3)+	0.03(1)i	0.60(9)+	9	0.6
$Mn(NO_3)_2$	(1)	79.20	110	0.06(6)i	0.03(3)i	0.01(1)i	0.09(10)+	10	0.4
	(10)	60.73	100	0.08(8)+	0.02(2)i	0.02(2)i	0.12(12)+	12	0.5
$Ni(NO_3)_2$	(1)	64.08	142	0.04(5)i	0.03(4)i	0.02(3)+	0.09(12)+	12	0.3
	(10)	59.67	116	0.09(10)+	0.05(6)+	0.01(1)i	0.15(17)+	17	0.6
$Pb(NO_3)_2$	(1)	54.67	110	0.07(8)+	0.04(4)+	0.01(1)i	0.12(13)i	13	0.5
	(10)	27.85	117	0.08(9)+	0.04(5)+	0.02(2)i	0.14(16)+	15	0.5
$Zn(NO_3)_2$	(1)	87.17	126	0.06(8)i	0.02(2)i	0.00(0)i	0.08(10)+	10	0.3
	(10)	84.00	110	0.06(6)i	0.03(3)i	0.01(1)i	0.09(10)+	10	0.4

[a] No of spots in parentheses;

[b] Statistical diagnoses according to Frei and Würgler (1988): += positive, -= negative, w= weak positive, i= inconclusive. m = multiplication factor. The conditional binomial test, one-sided, probability levels: $\alpha = \beta = 0.05$;

[c] Frequency of clone formation per 10^5 cells: *mwh* clones/wing /24 400 cell.

recombination. Twin spots, however, are thought to be the products of the mitotic recombination (Graf et al. 1984). The inhibition of DNA repair with metals was also reported. This is due to interaction of those metals with repair enzymes such as polymerases and ligases (Winder and Bonin 1993).

Finally, the results from this work showed that different metal compounds, of nitrate salts had a wide range of effect in terms of mutagenicity, $Co(N0_3)_2$ and $AgNO_3$ being the most effective genotoxic compounds.

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