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Comparative efficacy of chlorophyllin in reducing cytotoxicity of some heavy metals

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Summary. The potential of chlorophyllin in reducing clastogenicity was studied against two concentrations of each of three potent metallic clastogens (cesium chloride, mercuric chloride and cobalt chloride) in bone marrow cells of mice in vivo. The respective salts and chlorophyllin were administered orally to mice by gavaging in different combinations. Simultaneous administration of chlorophyllin with both concentrations of each salt reduced the clastogenic effects in the order Cs > Hg > Co. Chlorophyllin could not decrease the clastogenic effects when administered 2 h before the salts.

Key wods: Chlorophyllin - Clastogenicity - Bone marrow

Introduction

Cytotoxic effects of metals are receiving increasing attention because certain metals are potential mutagens, carcinogens and teratogens (see Sharma 1984; Sharma and Talukder 1987; Flessel et al. 1980). Of these, cesium (Cs) belonging to group I, mercury (Hg) to group II and cobalt (Co) to group VIII are three known clastogens (Ghosh et al. 1990; Das et al. 1982; Palit et al. 1991). Cesium as cesium chloride (CsCl) is considered to be the most toxic among the alkali chlorides and the amount in crops has increased considerably after the accidental nuclear fallout at Chernobyl (Bunzl and Kracke 1987; Jackson et al. 1987). Both inorganic and organic forms of mercury are known environmental clastogens (Das et al. 1982; Magos and Webb 1979). Though cobalt is essential for living organisms, cobaltous chloride ($CoCl_2$) is found to be toxic when given in excess (Domingo 1989; Palit et al. 1991). Exposure of different environmental genotoxic agents to the human population has generated considerable attention in the use of dietary supplements particularly plant and plant products, following the report of an inverse correlation between the consumption of vegetables and incidence of cancer (Hirayama 1981; Marshall et al. 1982; Peto et al. 1981; Winn et al. 1984). Efforts have been made in our laboratory to minimize the clastogenicity of certain toxic metals through simultaneous administration of certain plant products (Dhir et al. 1990; Agarwal et al. 1989). Chlorophyllin, a derivative of chlorophyll, exhibits antimutagenic activity against a variety of environmental and dietary complex mixtures (Ong et al. 1989). It is known to be an antioxidant (Sato et al. 1977), an effective photo-reducing agent (Brune and Pietro 1970) and has been used in gastro-intestinal medicine and for other therapeutic purposes (Imai et al. 1986).

The present work was undertaken to screen the action of chlorophyllin, a component of the plant pigment chlorophyll, against the clastogenic activity of three toxic metallic salts (CsCl, $HgCl_2$ and $CoCl_2$) in bone marrow cells of mice in vivo.

Materials and Methods

Laboratory bred Swiss albino mice (*Mus musculus*, $2n = 40$), about six-eight weeks old, weighing 25 ± 3 g, were procured from the departmental animal house, raised under standard laboratory conditions (temperature $20 \pm 3°$ C, relative humidity $50 + 15\%$ and photoperiod of 12 h). Standard pellet diet (Lipton India Limited) and distilled water were provided *ad libitum*. CsCl (Sisco Laboratories, India); $CoCl_2 \cdot 6H_2O$ (E. Merck, India) and $HgCl_2$ (Glaxo Laboratories, India) were dissolved in glass-distilled water corresponding to different fractions of the LD_{50} value (Table 1).

From the first set, three doses (0.77, 1.10 and 1.50 mg/kg body mass) of chlorophyllin sodium-copper salt (Sigma, St Louis, MO, USA) dissolved in glass-distilled water, were orally administered to the mice. The frequency of chromosomal aberrations remained almost at control level, indicating the non-toxic nature of the chemcial (Sen et al. 1991). In our experiments, the highest dose i.e. 1.50 mg/kg body mass was used. Cyclophosphamide (Sigma, St. Louis MO, USA), 25 mg/kg, was administered intraperitoneally as positive control.

For each experimental set, five mice were used. The mice were orally administered by gavaging (a) different metallic salts alone, (b) chlorophyllin alone; (c) chlorophyllin and the salts simultaneously and (d) chlorophyllin followed after 2 h by each salt in different sets according to the protocol given in Table 1. Control sets were maintained in glass-distilled water. All the animals were

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sacrificed after 24 h; 90 min prior to sacrifice, mice were injected intraperitoneally with 4 mg/kg body mass of colchicine (Sisco Laboratories, India). The animals were killed by cervical dislocation and bone marrow was flushed out and prepared for analysis of chromosomal aberrations following the usual hypotonic - acetic acid/ethanol fixative - Giemsa (E. Merck/India) staining schedule (Sharma and Sharma 1980). Slides were subsequently coded and scored blind for chromosomal aberrations; 50 well-scattered metaphase plates and a total of 250 plates/experimental set were scored. Individual aberrations were recorded separately. All aberrations (chromatid and chromosome gaps, chromatid and chromosome breaks and rearrangements) were considered equal, regardless of the number of breaks involved (Tice et al. 1987). The protocol for in vivo cytogenetic assay was followed strictly (Preston et al. 1987). A one-way ANOVA (Sokal and Rohlf 1981) followed by Duncan's new multiple-range test (Kotz and Johnson 1982) was carried out with the help of Harter's table (Harter 1960).

Table 1. Experimental procotol

Set	Doses administered (mg/kg body mass)			
	chemical alone		chlorophyllin 2 h before chemical	chlorophyllin + chemical simultaneously
I	Cyclophoshamide (positive control) 25		—	—
II	Distilled water (vehicle control)		—	
III	Chlorophyllin 1.5		—	—
IV	$CsCl$	A1 - 125	A2 1.5→125	A3 1.5+125
		B1 - 250	B2 1.5→250	B3 1.5+250
V	$HgCl_2$	C1 - 3	C2 1.5→ 3	C3 1.5+ 3
		D1 - 6	D2 1.5→ 6	D3 1.5+ 6
VI	$CoCl_2$	E1 - 20	E2 1.5→ 20	E3 1.5+ 20
		F1 - 40	F2 1.5→ 40	F3 1.5+ 40

Results and Discussion

In the present report, the degree of protection afforded by chlorophyllin, a known dietary inhibitor of mutagenesis, was tested against different doses of three metallic salts, cesium chloride, mercuric chloride and cobalt chloride. Chlorophyllin and the salts were administered orally to mice in different combinations, *viz.* singly, simultaneously or chlorophyllin followed by a metallic salt 2 h before the exposure. These different combinations were tested because compounds which afford beneficial effects under certain conditions may become ineffective when associated with other chemicals and their mode of administration. The end points screened in determining the relative clastogenecity and the degree of protection given, were the total chromosomal aberrations and the percentage of chromosomal aberrations (both including and excluding gaps) and breaks/cell (Table 2). Chlorophyllin (1.5 mg/kg body mass), when given alone, was not clastogenic as compared

Table 2. Data on chromosomal aberrations

Set	Total chromosomal aberrations					Percentae of total		Breaks/cell ($\bar{X}\pm$SEM)
	G′	G″	B′	B″	CR and others	including gap ($\bar{X}\pm$SEM)	excluding gap ($\bar{X}\pm$SEM)	
I	39	2	282	2	15	130 ±15.25	113.60±13.76	1.136±0.138
II	3	0	2	0	0	2.0 ± 0.89	0.80± 0.48	0.008±0.0017
III	8	1	2	0	0	4.4 ± 0.74	0.80± 0.48	0.008±0.0017
IV A1	12	0	33	0	17	24.8 ± 1.83	20.0 ± 0.79	0.2 ±0.004
A2	14	0	20	0	6	16.4 ± 2.14	10.4 ± 2.42	0.08 ±0.013
A3	25	1	9	0	1	14.4 ± 0.82	4.4 ± 0.66	0.04 ±0.004
B1	18	0	44	0	18	32.0 ± 1.26	24.8 ± 1.44	0.228±0.008
B2	10	0	31	0	23	25.6 ± 0.66	21.6 ± 1.03	0.204±0.013
B3	14	0	25	7	6	20.8 ± 2.00	15.2 ± 3.07	0.124±0.21
V C1	18	1	9	0	11	15.60± 1.46	8.0 ± 2.52	0.04 ±0.003
C2	17	1	6	0	6	12.00± 2.48	4.80± 1.19	0.028±0.0017
C3	17	1	3	0	0	8.40± 0.97	1.20± 0.48	0.012±0.0017
D1	19	5	12	0	10	18.4 ± 1.71	8.80± 1.02	0.052±0.008
D2	20	3	8	0	4	14.00± 1.99	4.80± 0.48	0.03 ±0.0013
D3	14	1	5	0	4	9.60± 1.46	3.60± 1.16	0.024±0.0031
VI E1	4	0	10	3	14	12.4 ± 1.16	10.8 ± 1.35	0.064±0.013
E2	10	1	11	0	10	12.8 ± 1.49	8.4 ± 1.72	0.056±0.009
E3	8	2	4	0	6	8.8 ± 1.85	4.2 ± 0.89	0.028±0.013
F1	11	1	14	2	21	19.6 ± 3.36	14.8 ± 2.86	0.072±0.013
F2	12	3	6	0	16	14.8 ± 1.49	8.8 ± 2.05	0.028±0.013
F3	8	3	6	0	15	12.8 ± 2.33	8.4 ± 1.16	0.05 ±0.013

G′, G″ = chromatid and isochromatid gaps; B′, B″ = chromatid and isochromatid breaks; CR = chromosomal rearrangements; others = polyploids, pulverised cells, pycnotic cells

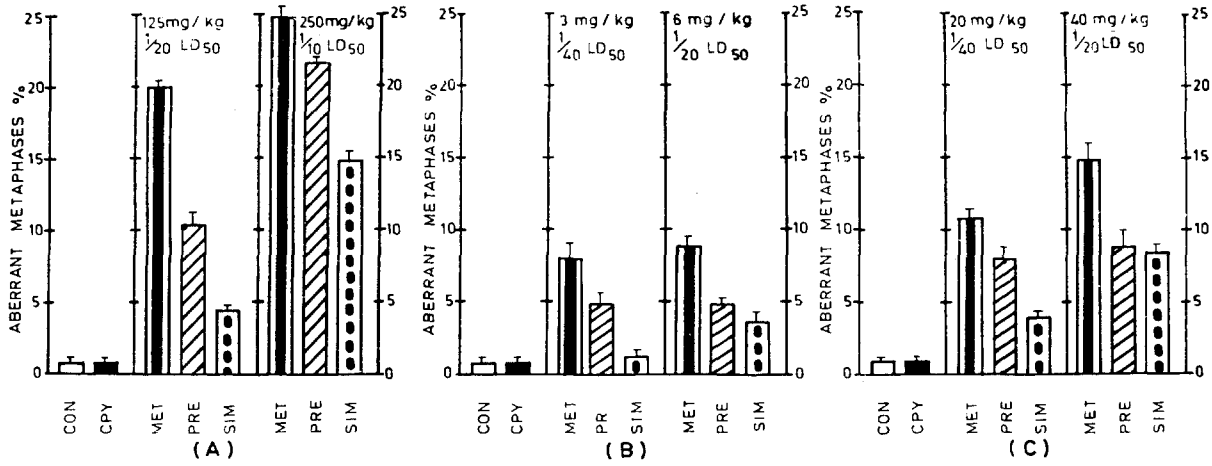

Fig. 1. Percentage of aberrant metaphases (excluding gaps) observed after administration of the metallic salt alone (MET) or given 2 h after administration of chlorophyllin (1.5 mg/kg body mass) (PRE) or both the salt and chlorophyllin administered simultaneously (SIM). Metallic salt: A cesium chloride; B mercuric chloride; C cobalt chloride. On the left of each panel are shown the aberrant frequencies of control animals (CON) and aberrations recorded after administration of chlorophyllin alone (CPY)

with the control kept on distilled water (Sen et al. 191). On the other hand, cesium chloride, mercuric chloride and cobalt chloride were highly clastogenic in both doses given (Ghosh A et al. 1990; Ghosh A. K. et al. 1991; Palit et al. 1990).

The decrease in frequencies of chromosomal aberrations was significantly higher with all the three metallic salts ($CsCl$, $HgCl_2$, $CoCl_2$) when chlorophyllin was administered simultaneously (Table 2). Chlorophyllin afforded protection against metal clastogenecity in a dose-dependent manner. Simultaneous administration of chlorophyllin with both doses of the metallic salts reduced clastogenicity in the following order: Cs > Hg > Co (Fig. 1).

When chlorophyllin was administered 2 h before the exposure to the salts, protection against the chromosomal damage induced by mercuric chloride and cobalt chloride was not effective. However, the protective action of chlorophyllin was significantly higher for both doses of cesium chloride.

The inhibitory action of chlorophyllin against the toxicity of a wide range of compounds such as nitropyrenes, nitroso compounds, flavonoids, aromatic amines and other polycyclic hydrocarbons has been well established (Ong et al. 1986). Chlorophyllin, chlorophyll *a* and chlorophyll *b* reduce the mutagenecity of cigarette-smoke condensate and benzo[*a*]pyrene (Terwel et al. 1985). Chlorophyllin is also reported to cause gene conversion and to reverse point mutation induced by physical and chemical mutagens on D_7 strains of *Saccharomyces cerevisiae* (Bronzetti et al. 1988). In another report, a positive correlation was established between the chlorophyll content of certain vegetables in minimizing the mutagenic activity of a number of chemicals (Lai et al. 1980; Barale et al. 1983). In our laboratory, chlorophyllin has shown antagonism against the clastogenic action of nicotine (Sen et al. 1991).

In the present study it was observed that chlorophyllin significantly reduced the chromosomal damage in mice bone marrow cells following exposure to three different metallic clastogens. The degree of protection varied with the mode of administration. Simultaneous administration of chlorophyllin with the metallic salts reduced chromosomal aberrations more effectively than giving chlorophyllin 2 h before the salts. These findings may be attributed to the known property of chlorophyllin to scavenge free radicals, to bind to the active groups of mutagens, to absorb or adsorb toxic compounds and to act as a membrane stabiliser (Hayatsu et al. 1988; Dhir 1989; Sharma 1990; Sato et al. 1984). Equitoxic concentration (1/20th LD_{50}) caused different levels of aberrations in the three metallic salts used. After substraction of the background aberration frequency and normalizing the data to 100% activity for the metal alone, activity of the metallic salt was determined for simultaneous application with chlorophyllin and when given 2 h after chlorophyllin. Inhibition of clastogenicity by chlorophyllin when given simultaneously was different for the different salts: $CoCl_2$ + chlorophyllin caused only 45.74% inhibition while $CsCl$ + chlorophyllin caused 81.25% inhibition. The activity of $HgCl_2$ was inhibited to 65% when given concomitantly with chlorophyllin.

The variation in the inhibitory action of chlorophyllin (1.50 mg/kg body mass in all the three sets) may be due to the structure and electronic configurations of the metals. The complex formation in binding of chlorophyllin to the metal ions can also influence the degree of protection against clastogenicity, through removal of the active metallic ion.

Since free phytol is readily absorbed, it was suggested that the phytyl-ester linkage of the chlorophyll molecule is resistant to the action of intestinal enzymes (see Sen et al. 1989). When administered 2 h earlier, chlorophyllin may be bound to other active principles in the gut, but when administered simultaneously with the chloride form of the metallic salts, it was able to inactivate the latter to a significant level. As there is an

inverse correlation between the intake of green fresh vegetables and the incidence of human gastro-intestinal cancer (Graham et al. 1978; Haenszel et al. 1976; Maugh 1982) and since clastogenicity is often associated with carcinogenecity, the present report showing significant inhibition of clastogenicity by chlorophyllin in vivo indicates the need for further extensive research on dietary intake of plant and plant products to overcome the adverse effects of metal pollution.

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