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Chromosome Damage Induced by Ferric Chloride in Human Peripheral Lymphocytes

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ABSTRACT The clastogenic activity of ferric chloride were observed on human lymphocyte culture *in vitro* after exposoure for 24 and 48 hrs. The results shows ferric chloride induces significantly high levels of chromosomal aberrations (%) and damaged cells (%) as compared to control. Period of exposure does not seem to affect the incidence to a significant level. Mitotic index however is reduced significantly as compared to control. The reduction being greater after exposure for 24 hours.

INTRODUCTION

Iron, a metal of great importance in man belonging to group VIIIB of the periodic table. It is considered as an essential element involved in a variety of essential biochemical reactions ranging from catalytic effects (e.g. in cytochromes, catalases, peroxidases and other mitochondrial enzymes) to involvement in DNA synthesis, oxygen transport and electron transfer (Bowen 1979; Zetkin and Schaldach 1974). The concentrations of iron exceeding 200 mg/day are considered toxic for man (Bowen 1979). World Health Organization study group determined that the maximal iron content of drinking water should not exceed 100µg/L.

The present work was undertaken to study clastogenecity of ferric chloride on human lymphocyte cultures *in vitro*.

MATERIALS AND METHODS

Chemical Treatment

Test Chemical: $FeCl_3$, Sigma St. Louis, MO, USA. Mol. wt. 162.20, CAS No. 7705-08-0, was tested for genotoxic effects and was dissolved in glass distilled water at a concentration of $3X10^{-5}$ (M).

Conditions and Duration of Exposure to the Test Compound: The ferric chloride solution was added to the culture at 24hr and 48hr after stimulation with phytohaemagglutinin, according to standard protocol (Preston et al. 1987). This is the optimum time for addition of the compounds. If the chemical is given in the G_0 phase, the response to PHA is affected leading to delay in mitosis. In addition, if the agent is short – lived, the cells are only exposed in G_0/G_1 phase which for many metallic compounds, is a relatively insensitive stage of the cell cycle.

Time of Fixation: Cells were harvested at 72 hrs for screening chromosomal aberrations following the standard protocol (Carrano and Natarajan 1988; Preston et al. 1987; Savage 1979) in order to avoid heterogenecity of cycle stage of the treated cells and to score only the first division mitotic cells.

Culturing Lymphocytes

Blood Samples: 10ml of human peripheral venous blood was collected aseptically in heparinized vials from six normal, healthy donors of both sexes between the ages of 20-45 years.

Culture Media and Conditions: Short term culture of lymphocytes was set up following the standard protocol (Natarajan and Obe 1980; Obe and Beek 1982; Preston et al. 1987; Sharma and Sharma 1994; WHO 1985). Lymphocytes in buffy coats were separated and approximately $1.0X10^6$ cells/ml medium were cultured. The culture medium consisted of 5ml RPMI 1640

(Sigma,USA) medium supplemented with 20% heat inactivated fetal calf serum (Sigma, USA), phytohaemagglutinin-M (0.2ml/5ml, GIBCO, Grand Island, NY, USA). Replicate cultures of each sample were maintained for each treatment / control, giving a total of six cultures for each blood sample. Culture tubes were incubated at 37±0.5^{0}C in a humidified atmosphere of 5±0.1% CO_2/95% air.

Metaphase Arrest: Colcemid (Sigma, USA) was added in a concentration of 0.5mg/ml, 2hr prior to the termination of cultures to obtain sufficient analysable metaphases.

Chromosome Preparation: Cells were collected by centrifugation, resuspended in a pre-warmed hypotonic KCl (0.075M) solution for 10-15 min and fixed in acetic acid- methanol (1:3, v/v), slides were prepared following air-drying (Rothfels and Siminovitch 1958) and stained with Giemsa (3%) for 20 min (Moorhead et al. 1960).

Analysis of Cells

Endpoints Screened: Mitotic index (MI), chromosomal aberration and percentage of aberrant cells(DC%), that is, cells having at least one aberration.

Number of Cells Screened: Mitotic index was determined as the percentage of dividing cells among 1000 nucleated cells in each set. For chromosomal aberration and percentages of aberrant cells, 100 well-scattered metaphase plates were screened per individual and 600 cells per dose point according to standard guidelines (Preston et al. 1987).

Chromosomal Aberration Analysis: Slides were scored blind and individual aberrations were recorded according to the standard protocol of Savage (1979) and Tice et al. (1987). All aberrations such as chromatid / chromosome breaks, dicentrics, rings, chromatid exchanges and polyploidy were considered to be equal. Chromatid/chromosome gaps were recorded but not included in the calculation.

Statistical Analysis: The data from the report sets were pooled and analysed statistically using one – way analysis of variance (ANOVA) (Sokal and Rohlf 1987) followed by Duncan's Multiple range test in order to compare the significance of different experimental sets(Kotz and Johnson 1982).

RESULTS

In the present study, iron compound *in vitro* is shown to have a direct effect on human lymphocytes following mitogenic stimulation.

Exposure to ferric chloride induces significantly high levels of chromosomal aberrations (%) and damaged cell (%) as compared to control (Table 1). Period of exposure does not seem to affect the incidence to a significant level. Mitotic index however is reduced significantly as compared to control the reduction being greater after exposure for 24 hours (Table 2).

DISCUSSION

Iron ions demonstrated cytotoxicity towards human embryonic cell (CLV102, Lu106) and human melanoma cells (Mel8) in a manner dependent on the concentration. The mixture of iron ions with ascorbic acid was less cytotoxic than when given alone (Marczewska et al. 2000).

The hydroxyl radicals produced by Fe metabolism is believed to be the cause of Fe toxicity. The hydroxyl radical is the most potent oxidizing agent that can exist in aqueous medium (Huebers 1991).

Table 1: Effect of ferric chloride on human chromosome *in vitro*

Set No.	*Treatment groups*	*Dose used*	*Exposure for*	*Chromosomal Aberrations*			*CA%*	*DC%*	*MI*
				B'	*B''*	*RR*	*(Mean±SD)*	*(Mean±SD)*	*(Mean±SD)*
							Excluding gaps		
I	Control (without metal)	-	-	1	-	-	0.167±0.408	0.167±0.408	5.6±0.962
II	Ferric chloride	3X10^{-5} (M)	24hrs	4	-	1	1.0±0.632	0.833±0.408	2.4±0.418
III	Ferric chloride	3X10^{-5} (M)	48hrs	5	-	-	0.833±0.408	0.833±0.408	2.7±0.274

B'= chromatid break, B''= isochromatid/chromosome break, RR= rearrangements, CA = chromosomal aberration, DC= damaged cell, MI=Mitotic Index.

Table 2: One way ANOVA table for chromosomal aberrations

Source of variation	*Df*	*SS*	*MSS*	*F*
SS between treatment groups	2	2.33	1.167	4.773*
SS within treatment groups	15	3.67	0.244	

* $p \leq 0.05$ significant

Duncan's Multiple range test

Treatment Groups	*Distilled water (vehicle control)*	*Ferric chloride exposure*	
		48hrs	24hrs
CA%(Mean)	0.167	0.833	1.0

Significant difference at $p \leq 0.05$

The role of free radicals and catalytic metal ions in human disease is well known (Halliwell and Gutteridge 1990; Lesnefsky 1994).

Histological analysis of liver tissue following hepatic arterial infusion of ferromagnetic particles in rabbit tumour model showed necrosis (Moroz et al. 2003).

In the present investigation, the degrees of clastogenic damage induced in the human lymphocyte culture were assessed in two separate experimental sets. Ferric chloride at a concentration of $3X10^{-5}$ was inoculated after 24hrs and 48hrs of culture in two respective sets. The resultant effects as shown by the frequency of chromosomal aberrations and mitotic index. (Table 2) shows that the chromosomal aberrations were statistically significant after ANOVA and from the Duncan's multiple range test it is observed that there is significant increase on chromosomal aberration than the control. The mitotic index shows a linear decrease when compared to control. But the chromosomal aberrations were decreased for longer period indicated repair in long term exposure.

The cellular requirement for iron is directly correlated with the cell type, the rate of cell growth, and the stage of cell differentiation. Maintenance of intracellular iron homeostasis demands the coordination of iron uptake, utilization, storage and in some cases release (Ponka et al. 1998). So, the clastogenecity of ferric chloride on lymphocyte culture although is well documented but needs further study on different cell type.

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