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Cytogenetic Alteration Induced by Nickel and Chromium in Human Blood Cultures and its Amelioration by Curcumin

M. V. Rao, D. D. Jhala, A. Patel and S. S. Chettiar

Cytogenetics Division, Zoology Department, University School of Sciences, Gujarat University, Ahmedabad 380 009, Gujarat, India
Telephone: 079-26302362, Fax: 079-26303196, E-mail: zooldeptgu@satyam.net.in

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ABSTRACT Tanners, welders and workers in various industries are exposed to acute and chronic toxicity of these heavy metals world wide. The present work is undertaken to evaluate the genotoxic effects of Ni and Cr at two different exposure intervals with a single dose and the amelioration of this toxicity using curcumin. Ni in form of nickel chloride (4.216×10^{-5}M) and Cr as potassium dichromate (1.36×10^{-6}M) were exposed for 24 and 69 hours to human blood lymphocyte cultures. The genotoxicity was measured by changes in acrocentric and telomeric association and C-anaphase. Results revealed a significant positive correlation between DNA damage and exposure time in Ni and Cr added cultures alone. Likewise it was also observed in cultures with combination of both pro-oxidants. Groups supplemented with curcumin (3.87×10^{-7}M) showed insignificant cytogenetic damage indicating its protective role which was calculated as percentage amelioration. Thus these data proved curcumin as a protective agent against Ni and Cr induced genotoxicity.

INTRODUCTION

Chromium (Cr) and nickel (Ni) are widely used industrial chemicals. The carcinogenic potential of metals is a major issue in defining human health risk from the exposure (Wenwei Hu et al. 2004). Welders, tanners and other occupationally exposed workers continue to suffer both acute and chronic health problems, which appear to be associated with exposure to Ni and Cr (Danadevi et al. 2004). Acute intoxications continue to be observed, consisting mostly of case reports of Cr intoxication related to bronchitis and pneumonitis from inhalation of welding fume (Coggon et al. 1994). Furthurmore, these metals are also known to induce SCEs and chromosomal aberrations in *in vitro* condition in human blood cultures (Rajvanshi et al. 2004).

Ni and Cr is known to induce DNA single-strand breaks (SSBs) in human white blood cells (Jelmert et al. 1994) as well as cytogenetic damage in peripheral blood lymphocytes of Ni and Cr -exposed workers (Stern et al. 1988; Knudsen et al. 1992; Danadevi et al. 2004). A recent hypothesis has postulated that oxidative stress may arise as a result of imbalance between oxidants and antioxidants, which contribute to the genotoxic effects of Ni and Cr exposure (Anatoly et al. 2002). A dose and time dependent increase in frequency of micronuclei was observed in human lymphocyte culture by Ni and Cr suggesting the genotoxic effects of the heavy metals (Rao et al. 2007). Contrarily there was also finding that showed average SCE frequency for welders significantly lower than that for the control group and DNA-protein cross-links were also higher among the welders than among the controls (Popp et al. 1991; Costa et al. 1993).

Curcumin, a major yellow pigment and active component of turmeric powder extracted from *Curcuma longa L.* (Gingiberaceae), has been shown to possess anti-inflammatory and anti-cancer activities (Baek et al. 2003). Dietary antioxidants protect laboratory animals against the induction of tumors by a variety of chemical carcinogens. Among possible mechanism of protection against chemical carcinogenesis could be mediated via-antioxidant-dependent induction of detoxifying enzymes (Iqbal et al. 2003). Shishodia et al. (2003) showed pre-treatment with curcumin abolished the cigarette smoke induced genotoxic manifestations. Leu and Maa (2002) advocated the chemopreventive and antitumorigenic effect of curcumin by elucidating the effect of curcumin on certain proteins like aryl hydrocarbon receptor, cytochrome P450, glu-

Author for Correspondence: Dr. M. V. Rao
Professor & Head Department of Zoology,
University School of Sciences,Gujarat University,
Ahmedabad 380 009, Gujarat. India
Telephone: 079-26302360 *Fax:* 079-26303196
E-mail: zooldeptgu@satyam.net.in

tathione S-transferase, serine/threonine kinases, transcription factors, cyclooxygenase, ornithine decarboxylase, nitric oxide synthase, matrix metalloproteinase and tyrosine kinases. Based on previous work in our laboratory, curcumin proved to be a strong protective agent against arsenic and fluoride induced genotoxicity (Rao and Tiwari 2006). Hence this study was undertaken to evaluate the role of this herbal product on heavy metal induced chromosomal aberrations in human blood cultures.

METHODOLOGY

***Subjects*:** Venous blood was collected from healthy non-smoking individual, aging from 20 to 25 years old, with their consent, in sterile heparinised syringes. Detailed information regarding the pre exposure of individual to any kind of drugs or habitual insult was taken.

***Peripheral blood lymphocyte culture (PBLC)*:** Cultures were set-up for each individual according to the standard protocol of Perry and Wolff (1974). Seven ml of RPMI –1640 (Himedia, Mumbai, pH 7.4) already supplemented with fetal calf serum (FCS) (Himedia, Mumbai) 10%, antibiotics (benzyl penicillin and streptomycin; Sarabhai Piramal Pharmaceuticals Ltd.,Vadodara.) and 100-µl phytohemagglutinin (PHA) (5mg/5ml distilled water; Sigma – Aldrich, USA), 0.5 ml of blood were added. Toxicants were added at 0 hour and at 45 hour of incubation so as to expose the culture for 69 hour and 24 hour respectively.

At the 69th hr., 30 µL of colchicines (1mg/5ml distilled water; Himedia, Mumbai) was added for 30 minutes so that those cells, which have entered the dividing M phase, would be arrested at the metaphase stage of cell division. At 72nd hour culture tubes were subjected to centrifugation at 1200 RPM for 15 minutes. The pellet obtained after centrifugation was treated with hypotonic solution (0.075 M KCl; Merck, Germany) for 20 min at 37°C. These cells were then fixed by 1:3 aceto:methanol fixative and profusely flushed, and were given at least three washes of fixative.

Slides were prepared from the cell suspension obtained after two washes with fixative. These slides were then stained with 2% Geimsa stain (Himedia, Mumbai) and observed under the microscope at 100× for scoring acrocentric association and C-anaphase in 100 metaphase plates.

Groups Studied: Cultures were divided into eight groups. Group I was test control. While in Group II curcumin alone was added (10µl/7ml media, 3.87 X 10^{-7}M; Himedia, Mumbai). Group III and Group IV were treated with Ni ($NiCl_2$, 4.216 X 10^{-5}M, Himedia, Mumbai) and Cr ($K_2Cr_2O_7$, 1.36 X 10^{-6}M; Himedia, Mumbai) respectively and Group V with a combination of the same concentrations of toxicants. Groups VI ,VII and VIII were curcumin supplemented with Ni, Cr and Ni + Cr. The positive control ethyl methyl sulfonate (EMS;Merck, Germany) was also done. Exposure intervals (24hr.and 69 hr.) remained same for all the test materials.

Analysis of Parameters: 100 Metaphase plates per group were analyzed under the microscope for association and C-anaphase. Percentage of amelioration was calculated by using the following formula: (Rao and Tiwari ; 2006)

(Pro-oxidant Group–Respective Antioxidant Group) / (Pro-oxidant Group – Control) ×100

Statistical Analysis: The data was analysed using student's t-test. Results are expressed as mean ± SE. All the treated groups were compared with the control group, and the curcumin-supplemented groups were compared with their respective pro-oxidant groups. P values less than 0.05 were considered to be significant.

RESULTS

After 24 hrs. of exposures, cultures treated with nickel and chromium alone did not exhibit remarkable alteration in the acrocentric association, telomeric association and C-anaphase as compared to control. Nickel + chromium added cultures showed a significant ($p<0.001$) increase in acrocentric and telomeric associations. However, cultures treated with combination of pro-oxidants showed no significant variation in C-anaphase values. Ni and Cr alone and in combination with curcumin supplementation revealed 80% to 85% amelioration (Tables 1, 3 & 5).

Prolonged exposure (69 hrs.) revealed that acrocentric association was significantly ($p<0.01$, $p<0.001$) increased in the cultures treated with Ni and Cr alone respectively (Table - 2), while telomeric association also exhibited a significant($p<0.05$) alteration (Table 4). However Ni and Cr treated cultures exhibited no alterations in C-anaphase values (Table 6). The combined groups (Ni + Cr) had a significant ($p<0.001$, $p<0.05$, $p<0.05$) increase in frequency of acrocentric association and telomeric associations as well as C-anaphase values

respectively (Tables 2, 4 & 6). Curcumin co cultures with pro-oxidants after 69 hrs. exposure induced a reduction in all these indices showing amelioration from 66% to 91% (Tables 2, 4 & 6).

DISCUSSION

The present data described the genotoxic effect of Ni and Cr in human blood cultures with

Table1: Effect of curcumin on Ni- and/or Cr-induced acrocentric association in 100

Groups	*DD*	*DG*	*GG*	*Multiple*	*Mean ± SE*	*T- test*	*% Amelieoration*
Control	7.8	6.8	4.2	5.6	24.4 ± 0.73	0	
Curcumin	7.6	7.0	4.4	5.4	24.4 ± 0.73	0	
Nickel	8.2	7.4	4.8	6.0	26.4 ± 1.34	1.309^{ns}	
Chromium	8.6	7.8	5.0	6.2	27.6 ± 2.23	1.361^{ns}	
Ni+Cr	11.4	9.8	6.0	7.6	34.8 ± 1.53	6.102*	
Ni+Cur	8.0	6.8	4.6	5.4	24.8 ± 1.05	0.312 ^{ns}	80.0
Cr+Cur	8.0	7.0	4.4	5.6	25.0 ± 1.35	0.392 ^{ns}	81.3
Ni+Cr+Cur	8.0	7.0	5.0	6.0	26.0 ± 1.57	0.922 ^{ns}	84.6

metaphase plates in Human lymphocyte cultures after 24-hr exposure (n=5)
*p<0.001, ns non significant

Table2: Effect of curcumin on Ni- and/or Cr-induced acrocentric association in 100 metaphase plates in human lymphocyte cultures after 69-hr exposure (n=5)

Groups	*DD*	*DG*	*GG*	*Multiple*	*Mean ± SE*	*T- test*	*% Amelieoration*
Control	8.0	6.4	4.2	5.6	24.2 ± 0.70	0	
Curcumin	7.8	6.8	4.4	5.6	24.6 ± 0.73	0.395 ^{ns}	
Nickel	9.8	7.4	5.2	7.4	29.8 ± 1.55	3.288*	
Chromium	11.0	8.0	6.4	8.2	33.6 ± 1.64	5.276**	
Ni+Cr	13.0	9.2	7.2	9.0	38.4 ± 1.27	9.806**	
Ni+Cur	8.2	6.6	4.4	5.8	25.0 ± 0.90	0.699 ^{ns}	85.7
Cr+Cur	9.0	7.4	4.4	5.8	26.6 ± 1.23	1.698 ^{ns}	74.4
Ni+Cr+Cur	11.0	7.0	5.0	6.0	29.0 ± 1.32	3.216*	66.2

*p<0.01, **p<0.001, ns non significant

Table 3: Effect of curcumin on Ni- and/or Cr-induced telomeric association in 100 metaphase plates in human lymphocyte cultures after 24-hr exposure (n=5)

Groups	*Chromatid*	*Chromosomal*	*Mean ± SE*	*T- test*	*% Amelieoration*
Control	9.4	3.6	13.0 ± 0.76	0	
Curcumin	9.6	3.6	13.2 ± 0.50	0.217 ^{ns}	
Nickel	10.0	3.8	13.8 ± 1.41	0.496 ^{ns}	
Chromium	10.2	4.0	14.2 ± 2.35	0.484 ^{ns}	
Ni+Cr	13.4	5.4	18.8 ± 1.20	4.059*	
Ni+Cur	9.6	3.6	13.2 ± 1.05	0.153 ^{ns}	75.0
Cr+Cur	9.8	3.4	13.2 ± 1.25	0.136 ^{ns}	83.3
Ni+Cr+Cur	10.2	3.4	13.6 ± 1.53	0.35 ^{ns}	89.7

*p<0.001, ns non significant

Table 4: Effect of curcumin on Ni- and/or Cr-induced Telomeric Association in 100 metaphase plates in Human lymphocyte cultures after 69-hr exposure (n=5)

Groups	*Chromatid*	*Chromosomal*	*Mean ± SE*	*T- test*	*% Amelieoration*
Control	9.4	3.4	12.8 ± 0.72	0	
Curcumin	9.6	3.4	13.0 ± 0.72	0.1968 ^{ns}	
Nickel	11.0	4.0	15.0 ± 1.55	1.2883 ^{ns}	
Chromium	12.4	5.4	17.8 ± 1.28	3.40785*	
Ni+Cr	13.8	6.2	20.0 ± 1.86	3.60321*	
Ni+Cur	9.4	3.6	13.0 ± 1.04	0.15847 ^{ns}	91
Cr+Cur	10.0	3.4	13.4 ± 1.30	0.40372 ^{ns}	88
Ni+Cr+Cur	10.2	4.0	14.2 ± 1.38	0.89891 ^{ns}	80

*P<0.005, ns non significant

Table 5: Effect of curcumin on Ni- and/or Cr-induced C-anaphase in 100 metaphase plates in human lymphocyte cultures after24-hr exposure (n=5)

Groups	*C- Anaphase*	*Meanstd error*	*T- test*	*% Amelieoration*
Control	0.6	0.6 ± 0.40	0	
Curcumin	0.6	0.6 ± 0.24	0	
Nickel	1.0	1.0 ± 0.63	0.5345 ^{ns}	
Chromium	1.4	1.4 ± 0.74	0.9428 ^{ns}	
Ni+Cr	2.0	2.0 ± 0.70	1.72328 ^{ns}	
Ni+Cur	0.8	0.8 ± 0.37	0.36515 ^{ns}	50
Cr+Cur	1.0	1.0 ± 0.40	0.70711 ^{ns}	50
Ni+Cr+Cur	1.2	1.2 ± 0.58	0.84853 ^{ns}	57

ns non significant

Table 6: Effect of curcumin on Ni- and/or Cr-induced C-anaphase in 100 metaphase plates in human lymphocyte cultures after 69-hr exposure (n=5)

Groups	*C- Anaphase*	*Mean ± SE*	*T- test*	*% Amelieoration*
Control	0.8	0.8 ± 0.58	0	
Curcumin	0.6	0.6 ± 0.40	0.28284 ^{ns}	
Nickel	2.4	2.4 ± 0.93	1.46059 ^{ns}	
Chromium	3.6	3.6 ± 1.03	2.36643 ^{ns}	
Ni+Cr	4.2	4.2 ± 1.74	1.84936 *	
Ni+Cur.	1.2	1.2 ± 0.58	0.48507 ^{ns}	75.0
Cr.+Cur	1.4	1.4 ± 0.61	0.71307 ^{ns}	78.5
Ni.+Cr.+Cur.	1.4	1.4 ± 0.51	0.7746 ^{ns}	82.3

*p<0.05, ns non significant

respect to acrocentric satellite association, telomeric association and C-anaphase. It is known that nickel (II) and chromium (III) enter the cell directly while Cr (VI) penetrates only by means of sulfate ion channel. After entering into the cell it converts into the stable form Cr(III) (Kerger et al. 1997). These Ni(II) and Cr(III) ions accelerate the formation of the free radicals which cause lipid peroxidation as well as DNA damage. These free radicals also inhibit the DNA repair enzymes followed by cell cycle regulatory enzymes proteins leading to low cell proliferation. Chromium and nickel compounds further interact with DNA repair processes that lead to an enhancement of genotoxicity in combination with a variety of DNA damaging agents (Klein et al. 1991; Kasprzak 1991). Millosevic et al. (2005) showed that the newborns exposed to environmental pollutant containing Ni and Cr illustrate high frequency of micronuclei, which supports our finding . Thus both metal compounds are potential in bringing about cytogenetic attrition inducing genotoxicity.

Telomeres protect the end of the chromosome against degradation, inappropriate recombination and fusion events. Telomeric shortening might lead to sticky ends which join to result into nondisjunction in forth coming cell division (Blackburn 2001). Telomeric sequences are also lost with cell division alongwith other causes including oxidative stress (Von et al. 1995; Andrei and Peter 2003). In addition to loss of telomeric DNA with replication, oxidative damage of telomeric DNA is an important cause of the telomere shortening followed by telomeric association in normal human somatic cells (Saretzki 2002; Collins and Mitchell 2002). This acceleration was attributed to the enhanced induction of telomeric single strand breaks by free radicals, leading to the loss of the distal fragments of telomeric DNA following replication (Sitte et al. 1998). Other studies revealed that telomeric DNA is a preferential target for oxidative damage (Henle et al. 1999; Oikawa and Kawanishi 1999). Liu et al. (2002) reported arsenic induces telomeric association by enhancing the oxidative stress in the cells. Similarly our results supported an increased telomere association probably due to telomeric shortening in cultures added with prooxidants.

The fission of two or more nucleoli would tend to stretch mechanically the nucleolar forming chromosome segment with obvious risk of breakage. If breaks occur in more than one of the chromosomes involved the closeness of the broken ends would predispose to translocations and

the acrocentirc satellite association (SA) would thus be active also in the origin of translocation between satellite chromosomes. A high incidence of SA as a result of any chemical insult has often been considered as causative to an increased tendency of nondisjunction and thus to the induction of D and G trisomies or structural aberration. The elevated frequency in acrocentric satellite association in our study, is attributed to the ability of pro-oxidants to induce DNA damage.

The appearance of C-anaphase indicated the premature onset of anaphase in Ni and Cr arrested mitotic lymphocyte cells. Huang et al. (1998) demonstrated that heavy metals could interfere with the functions of mitotic spindles by attenuating the dynamics of tubulin and hence, induce mitotic arrest in HeLa cells. In our study, thses data demonstrated that Ni and Cr also likely block the onset of metaphase to anaphase transition, this inhibition could be due to spindle inactivation in pro-oxidant treated mitotic cells. Thereafter, the defective bipolar spindles would not allow completion of normal chromosome segregation and cytokinesis. To trigger the transition of metaphase into anaphase, the exit from mitosis and cytokinesis is generally known to be via the activation of APC/C, a multi-subunit complex with ubiquitin-ligase activity (Townsley et al. 1998) , and by its association with activator proteins p55cdc20 or cdh1 to form the APC/C^{cdc20} or APC/C^{cdh1} complex (Burke 2000) . The APC/C^{cdc20} complex is required for the onset of anaphase by triggering the proteolytic degradation of Pds1 (Nasmyth et al. 2000), whereas the APC/C^{cdh1} complex is needed for cytokinesis and mitotic exit by complete degradation of mitotic cyclins (Visintin et al. 1997). The sequential activation of APC/C^{cdc20} and APC/C^{cdh1} plays a key role in establishing the temporal order of cell cycle control (Nigg 2001). Ni and Cr possibly hampered cell cycle regulation by directly interacting with these proteins and inducing an imbalance in cell cycle. Disruption of the temporal order of cell cycle progression might severely alter chromosome segregation and lead to loss of genomic integrity (Nigg 2001; Cerutti and Simanis 2000; Luch 2002).

Curcumin supplementation brought about protective effect on nickel and chromium induced genotoxicity, as the values of acrocentric association, telomeric association and C-anaphase were comparable to control cultures. Its amelioration effect is related to possession of parahydroxyl groups, which have antioxidant activity while other reactive groups like keto and double bonds of curcumin have anti mutagenic effects. Hence this herbal product scavenges and neutralizes free radical generated during toxic reactions in response to the chemical insult, by breaking their subsequent oxidative chain reactions (Das and Das 2002; Rajvanshi et al. 2004). In conclusion, curcumin proved to be a very strong protective agent against Ni and Cr induced genotoxic indices in our study.

CONCLUSION

To conclude, our study suggests that the curcumin had the potential ameliorative effect on nickel and chromium exerted genotoxicity *in vitro*.

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