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A Report on Myelosuppressive and Lymphopenic Effects of Hexavalent Chromium in a Murine Model

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Abstract

Both leucocyte and erythrocyte counts of blood sharply decreased in Swiss mice after 2 weeks of intraperitoneal treatment with potassium dichromate (4 mg/Kg for 5 days per week). A marked neutropenia along with a relative rise in lymphocyte count were also noted. Chromosomal abnormalities (chromatid break, ring chromosome formation and aneuploidy) and micronucleus formation were observed in both lymphopoietic and myelopoietic tissues (spleen and bone marrow, respectively). The overall findings indicate that a cytotoxic potential of hexavalent chromium may result in myelosuppression, lymphopenia as well as suppression of erythropoiesis. However, a higher incidence of chromosomal abnormalities and micronucleated cells in the bone marrow than in the spleen suggests hexavalent chromium to be relatively more myelosuppressive than lymphopenic in action.

Key Words: Chromium, Spleen, Bone marrow, Lymphocyte, Neutrophil

Introduction

The heavy metal chromium in the form of its hexavalent salts has long been marked as an environmental pollutant by toxicologists as well as physiologists. Hexavalent chromium has been identified as a potent inorganic carcinogen [1, 2]. It has also been known to exert considerable hepatotoxic and nephrotoxic actions in animal models [3, 4]. Besides, hexavalent chromium has been reported to adversely affect the reproductive function in both sexes of laboratory mouse [5].

Hexavalent chromium has also been known to possess marked clastogenic (chromosome damaging) as well as cytotoxic potentials. Hexavalent chromium salts (potassium dichromate and potassium chromate) were found to produce chromosomal aberrations in (i) human leucocytes grown *in vitro* [6], (ii) phytohaemagglutinin-stimulated human lymphocytes reared *in vitro* [7] and (iii) Chinese hamster ovarian cells cultured *in vitro* [8]. Chromosomal aberrations were also reported in the bone marrow of mice treated with hexavalent chromium along with drinking water [9]. Hexavalent chromium at concentrations above 2.5 µM has been found to affect the viability of mouse peritoneal macrophages *in vitro* in a concentration-dependent manner [10]. Recently, it has been reported that hexavalent chromium, at concentrations above 1 µM, reduces the viability of human macrophages *in vitro* [11]. Another recent report indicates that potassium dichromate, when added in soil (250 mg/Kg), may exert a cytotoxic action in germinating seeds and growing seedlings of plants [12].

In view of the aforementioned reports on clastogenic and cytotoxic potentials of hexavalent chromium, the present authors have been interested to make a preliminary investigation on whether chromium is likely to suppress *in vivo* the production of cells concerned with immunity, in a murine model, or not. Lymphocytes of vertebrate body are concerned with elaboration of humoral immune response while neutrophils are concerned with innate immunity involving phagocytosis [13]. The spleen is a major lymphopoietic organ while the bone marrow is the site for myelopoiesis (production of granulocytes) in mammalian body [14]. In the present study, the probable immunosuppressive (myelosuppressive and lymphopenic) potential of hexavalent chromium has been evaluated through examination of (i) total count of leucocytes of blood, (ii) differential counts of all kinds of leucocytes of blood, (iii) chromosomal abnormalities in lymphopoietic and myelopoietic tissues (spleen and bone marrow, respectively) and (iv) micronucleus formation in lymphopoietic and myelopoietic tissues in a murine model. Simultaneously, erythrocyte count of blood has also been recorded to assess whether chromium also suppresses erythropoiesis in the bone marrow, or not.

The rationale behind undertaking the present study is that hexavalent chromium appears to be a major environmental pollutant in the tannery-belt of the Greater Kolkata. An indiscriminate discharge of a huge quantity of a chromium-rich effluent by several tanneries in the Bantala Leather Complex, where the construction of suitable chromium-treatment plants is still pending, is likely to adversely affect the health of the tannery workers as well as the people living around the tanneries [15]. It is, therefore, necessary to obtain as much information as possible on the adverse physiological effects of chromium.

Materials and Methods

Adult (8 weeks old), inbred and healthy, male, albino mice of the Swiss strain were divided into control and experimental groups. Only male mice were taken in analogy with the fact

that the tannery workers of our state are generally males. The mice were housed in a few polypropylene cages with saw-dust for bedding, in a well-ventilated and well-lit animal room of the Department of Zoology, Presidency University, Kolkata. The mice were provided with food pellets and filtered tap-water ad libitum. Animal care was taken as properly as possible under the regular supervision of an Institutional Animal Ethical Committee whose registration number with the Committee for the Purpose of Control and Supervision on Experiments on Animals, Ministry of Environment and Forests, Government of India, is 796/03/ac/CPCSEA.

The experimental mice were intraperitoneally injected with an aqueous solution of potassium dichromate (K2Cr2O7) at a dose of 4 mg/Kg body weight for 5 consecutive days per week, for a total period of 2 weeks. The selected dose of K2Cr2O7 was nearly one-tenth of its LD50 value (39 mg/Kg) for mice [4]. The control mice were injected with physiological saline (0.9%) for a similar tenure.

After 1 and 2 weeks of chromium treatement, 6 experimental and 6 control mice were anaesthetised under ether vapour and blood samples were collected inside heparinised vials by cardiac puncture. Total counts (TC) of erythrocytes and leucocytes were determined by refined visual methods using a haemocytometer having an improved Neubauer counting chamber [16]. Differential counts (DC) of leucocytes were determined from microscopic examination of blood smears stained with 10% Giemsa's stain buffered to pH 7.0 with 0.1 M phosphate buffer [16].

Chromosomal preparations were made from both lymphopoietic and myelopoietic tissues (spleen and bone marrow, respectively) of both experimental and control groups of mice by using the conventional flame-drying technique [17]. Micronucleus test was carried out on both spleen and bone marrow cells of the animals following the popular citrate-Giemsa method [18].

Results and Discussion

At the end of the first week of $K_2Cr_2O_7$ treatment, TC of erythrocytes was considerably decreased in the experimental mice, as compared to that in the controls ($p < 0.001$). However, at this stage, both TC and DC of leucocytes did not show any significant difference between the control and the experimental groups of mice ($p > 0.05$ in either case). At the end of the second week of treatment, TC of erythrocytes was found to be further decreased. Moreover, TC of leucocytes was noted to be markedly reduced in the experimental mice, as compared to that in the controls ($p < 0.001$). Interestingly, DC of leucocytes revealed that the count of lymphocytes was considerably higher in the experimental mice than in their control counterparts ($p < 0.001$) while the count of neutrophils was significantly lower in the former than in the latter ($p < 0.001$). The counts of blood cells in the experimental and the control mice, as recorded during the present study, have been summarized in Table 1.

Chromosomal abnormalities were occasionally observed in both spleen and bone marrow of the experimental mice. A metaphase plate of a control mouse showed 40 telocentric chromosomes, each with two chromatids of equal length. On the other hand, one or more chromatid breaks, formation of one or more ring chromosomes and aneuploidy (loss of one or more chromosomes) were occasionally observed in the metaphase plates of the experimental mice (Fig. 1 and Fig. 2). Chromosomal abnormalities were more frequently encountered in the bone marrow than in the spleen, at the end of either 1 or 2 weeks of $K_2Cr_2O_7$ treatment. Moreover, as summarized in Table 2, the overall percentage of chromosomal abnormalities was found to be higher in both the tissues, after 2 weeks than after 1 week of $K_2Cr_2O_7$ treatment.

Micronuclei were seldom observed in either the spleen cells or the bone marrow cells of any control mouse. On the other hand, a progressive increase in the incidence of micronuclei in both spleen cells and bone marrow cells was noted in the experimental mice after 1 and 2 weeks of $K_2Cr_2O_7$ treatment (Table 3). A micronucleus appeared as a small and rounded body (1-2 μm in diameter) lying in the cell cytoplasm and staining violet as intensely as the main nucleus of a cell (Fig. 3 and Fig. 4). The percentage of micronucleus-bearing cells was found to be higher in the bone marrow than in the spleen after 1 as well as 2 weeks of $K_2Cr_2O_7$ treatment.

The finding of a progressive decrease in TC of erythrocytes in the $K_2Cr_2O_7$-treated mice very strongly indicates a suppressive action of hexavalent chromium on erythropoiesis. Moreover, a marked reduction in TC of leucocytes after 2 weeks of tretment indicates that hexavalent chromium also exerts a suppressive action on leucopoiesis in the murine model. However, the suppressive action on leucopoiesis is initiated somewhat later than that on erythropoiesis.

After 2 weeks of $K_2Cr_2O_7$ treatment, TC of both leucocytes and erythrocytes are found to be sharply decreased along with a marked fall in DC of the neutrophils (which causes and apparent and relative rise in DC of the lymphocytes). The findings distinctly indicate that hexavalent chromium exerts a relatively stronger suppressive action on erythropoiesis and myelopoiesis in the bone marrow than on lymphopoiesis in the spleen of the murine model.

The finding of various kinds of chromosomal abnormalities in both spleen and bone marrow of the experimental mice is indicative of a strong cytotoxic potential of chromium. An occasional formation of micronucleus also provides a similar indication; a micronucleus has long been known to originate due to condensation of broken chromosomal parts dislodged to the cytoplasm from the nuclei of the cells [19]. The cytotoxic potential of hexavalent chromium may be responsible for considerable suppression of lymphopoiesis in the spleen and both erythropoiesis and myelopoiesis in the bone marrow in the murine model under study.

Table 1. Blood cell counts (mean ± s. d.) in $K_2Cr_2O_7$-treated and control Swiss mice (6 mice per group).

Blood cells	Groups of mice		Groups of mice	
	1 week after treatment	Respective controls	2 weeks after treatment	Respective controls
Erythrocyte (million/c.mm)	5.01 ± 0.33*	5.64 ± 0.27	4.76 ± 0.23*	5.66 ± 0.35
Leucocyte (thousand/c.mm)	6.47 ± 0.43	6.55 ± 0.51	4.91 ± 0.28*	6.43 ± 0.34
Lymphocyte (%)	75.50 ± 2.99	76.83 ± 2.11	84.33 ± 2.87*	75.83 ± 2.41
Neutrophil (%)	19.67 ± 2.87	17.67 ± 2.43	10.67 ± 3.14*	19.00 ± 3.26
Monocyte (%)	3.50 ± 0.50	3.67 ± 0.74	3.67 ± 0.74	3.67 ± 0.74
Eosinophil (%)	1.33 ± 0.47	1.83 ± 0.69	1.33 ± 0.47	1.50 ± 0.50

*Significantly different from respective controls ($p < 0.001$).

Table 2. Chromosomal abnormalities (% of 50 metaphase plates examined per group) in $K_2Cr_2O_7$-treated and control Swiss mice

Tissue and chromosomal abnormality	Groups of mice		Groups of mice	
	1 week after treatment	Respective controls	2 weeks after treatment	Respective controls
(A) Spleen :				
(i) chromatid break	12	0	16	0
(ii) ring chromosome	4	0	4	0
(iii) aneuploidy	4	0	4	0
(B) Bone marrow :				
(i) chromatid break	18	0	22	0
(ii) ring chromosome	4	0	6	0
(iii) aneuploidy	4	0	6	0

Table 3. Micronuclei formation (% of 200 cells examined per group) in $K_2Cr_2O_7$-treated and control Swiss mice

Tissue	Groups of mice		Groups of mice	
	1 week after treatment	Respective controls	2 weeks after treatment	Respective controls
Spleen	2.0	0	3.5	0.5
Bone marrow	3.5	0.5	5.5	0.5

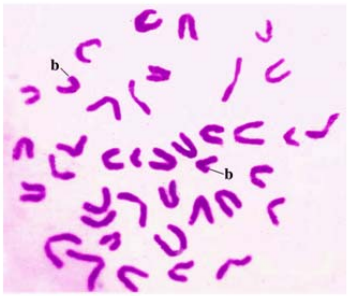

Fig. 1. A metaphase plate from spleen of a mouse (after 2 weeks of $K_2Cr_2O_7$ treatment), showing aneuploidy (chromosome number = 39) and chromatid breaks (b)

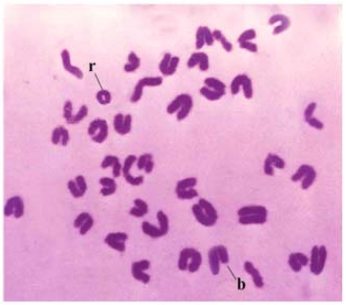

Fig. 2. A metaphase plate from bone marrow of a mouse (after 2 weeks of $K_2Cr_2O_7$ treatment), showing aneuploidy (chromosome number = 39), chromatid break (b) and ring chromosome (r).

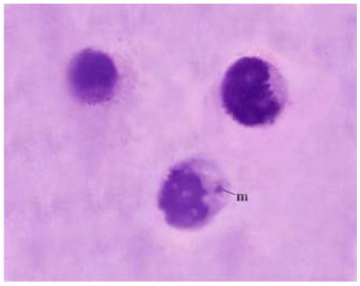

Fig. 3. Micronucleus (m) formation in a spleen cell of a mouse (after 2 weeks of $K_2Cr_2O_7$ treatment).

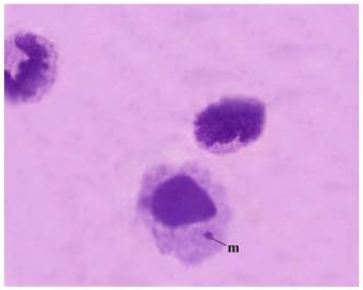

Fig. 4. Micronucleus (m) formation in a bone marrow cell of a mouse (after 2 weeks of $K_2Cr_2O_7$ treatment).

Conclusion

It may be concluded from the present study that hexavalent chromium exerts a marked cytotoxic action on lymphopoietic as well as myelopoietic tissues (spleen and bone marrow, respectively) of a murine model, resulting in marked leucopenia (along with a markedly decreased erythrocyte count). The leucopenic effect is, on the other hand, indicative of a probable immunosuppressive effect of chromium. However, a higher incidence of chromosomal abnormalities and micronucleated cells in the bone marrow than in the spleen indicates that hexavalent chromium is relatively more myelosuppressive than lymphopenic in action.

Finally, an extrapolation of the findings of the present study in a murine model hints to the necessity of periodic monitoring of blood cell counts in the tannery workers of our state, who are continually getting exposed to hexavalent chromium on account of their occupation.

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