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REVIEW ARTICLE

Adverse hematological effects of hexavalent chromium: an overview

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ABSTRACT

Workers of tanneries, welding industries, factories manufacturing chromate containing paints are exposed to hexavalent chromium that increas¬es the risk of developing serious adverse health effects. This review elucidates the mode of action of hexavalent chromium on blood and its adverse effects. Both leukocyte and erythrocyte counts of blood sharply decreased in Swiss mice after two weeks of intraperitoneal treatment with Cr (VI), with the erythrocytes transforming into echinocytes. The hexavalent chromium in the blood is readily reduced to trivalent form and the reductive capacity of erythrocytes is much greater than that of plasma. Excess Cr (VI), not reduced in plasma, may enter erythrocytes and lymphocytes and in rodents it induces microcytic anemia. The toxic effects of chromium (VI) include mitochondrial injury and DNA damage of blood cells that leads to carcinogenicity. Excess Cr (VI) increases cytosolic Ca^{2+} activity and ATP depletion thereby inducing eryptosis. Se, vitamin C, and quercetin are assumed to have some protective effect against hexavalent chromium induced hematological disorders.

KEY WORDS: hexavalent chromium; haematological disorders; haemolytic anaemia, chromium in blood

Introduction

Heavy metals are ubiquitous and persistent environmental pollutants that are generally introduced into the environment through anthropogenic activities (Achal *et al.*, 2011). Chromium (Cr) is one of the toxic environmental pollutants released in the environment due to its wide use in industries such as tanning, corrosion control, plating, pigment manufacture and nuclear weapon production (Singh *et al.*, 2013). The extensive industrial usage of Cr compounds and subsequent release of effluents, without proper treatment in the environment, contaminates the ecosystem and causes remarkable health problems.

Chromium exists in several oxidation states, with the most stable forms being trivalent chromium [Cr (III)] and hexavalent chromium [Cr (VI), chromates] species, with different chemical characteristics and biological effects (Nath *et al.*, 2009). Of these two, water soluble hexavalent chromium is extremely irritating and toxic to tissues of the human body as the solubility promotes active transport of chromate across biological membranes (Cervantes *et al.*, 2001). Once internalized by cells, it exhibits a variety of toxic, mutagenic, and carcinogenic effects (Ackerley *et al.*, 2006). Tannery workers, welders, workers of pigments and paint industries are regularly exposed to hexavalent chromium that increases the risk of developing serious adverse health conditions. Occupational exposure to hexavalent chromium may induce a number of hematological disorders characterized by abnormal features of blood cells. The cytotoxic nature of Cr (VI) is well evident from the results of *in vitro* and *in vivo* studies in laboratory animal models and is comparable to that of the human system.

The present overview focuses on the morphological, biochemical and physiological effects of hexavalent chromium on mammalian blood, on the basis of observational evidence from altered hematological profiles of tannery workers and experimental animals.

Biomedical profiles

Workers in industries, with exposure primarily to Cr (VI) via inhalation of aerosols, are at the greatest risk of suffering adverse health effects. Repeated exposure to Cr (VI) compounds causes similar effects in both humans and laboratory animals, which include irritant and inflammatory effects on the respiratory system and immunological changes such as increased serum immunoglobulin and white blood cell count, as well as changes in the activities of alveolar macrophages and spleen lymphocytes (IPCS,

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2006; ECB, 2005). Adverse health effects associated with longterm Cr (VI) exposure include occupational asthma, eye irritation and damage, perforated eardrums, respiratory irritation, kidney damage, liver damage, pulmonary congestion and edema, upper abdominal pain, nose irritation and damage, respiratory cancer, skin irritation, as well as erosion and discoloration of teeth. Some workers can also develop an allergic skin reaction, called allergic contact dermatitis, resulting from handling liquids or solids containing Cr (VI). Furthermore, contact with skin wound can lead to formation of crusted, painless lesions showing a pitted ulcer covered with fluid (De Flora, 2012), called chrome ulcer.

Increased blood chromium level after total hip replacement using metal-metal pairings cause the release of metal ions of the alloys (Schaffer & Pilger, 1999), which in turn produces a number of toxic manifestations. Short-term peak inhalation exposures to acute high concentration of Cr (VI) may block the reducing abilities of the body and can cause serious health effects (ATSDR, 2000), like respiratory irritation, asthma and gastrointestinal irritation. This picture is in agreement with the results of Langård and Nordhagen (I980) observed in laboratory animals subjected to whole-body exposure at high concentrations of hexavalent chromium. Occurrence of microcytic, hypochromic anemia was reported in several recent animal studies with chromium (VI) compounds. Hematological findings in humans exposed to lethal doses of chromium (VI) compounds were difficult to interpret in the context of multiple systemic effects leading to death, following hemorrhage.

Recent studies indicate a biological relevance of non-oxidative mechanisms in Cr(VI) carcinogenesis (Zhitkovich *et al.,* 2001). The toxicity of chromium within the cell may result from damage to cellular components during the hexavalent to trivalent chromium reduction process by generation of free radicals, including DNA damage (ATSDR, 2000) Cr(VI). Once it is reduced intracellularly, it produces various forms of DNA damage including DNA interstrand cross links, DNA-protein crosslink, DNA strand breaks, and Cr-DNA adducts. This DNA damage presumably accounts for the observed functional changes in DNA replication and transcription, which may be crucial to the carcinogenicity of chromium (VI) compounds.

Toxicological profiles

In tanning industry, the chromium concentration in the exhaust chromium liquor volume discharged from the tanning process ranges from 1,500–5,000 mg/L, which by mixing with other effluent streams from the tannery process become 100–300 mg/L. As per international and Indian standards, wastewater effluents should not exceed 0.05 mg/L Cr(VI) (Jabari *et al.*, 2009, Mythili & Karthikeyan, 2011).

According to Dana Devi *et al.* (2004) a person spends, on average, one-third of life at the workplace. Hence, the toxic effects of Cr (VI) in the blood of victims of occupational exposure are well reflected in their hematological conditions. Chromium concentration in erythrocytes was about two times higher in electroplating workers (4.41 µg/L) than that in control subjects (1.54 µg/L) (Zhang *et al.*, 2011). Halasova *et al. (*2012) reported the level of chromium in the blood of welders regularly exposed to Cr (VI) to range between 0.032 and 0.182 µmol l^{-1}, which was significantly higher than in controls. The study of Sellappa *et al. (*2011) suggested that chronic occupational exposure to Cr (VI) during welding could lead to increased levels of DNA damage and repair inhibition, which were depicted by the genotoxicity in lymphocytes of these welders.

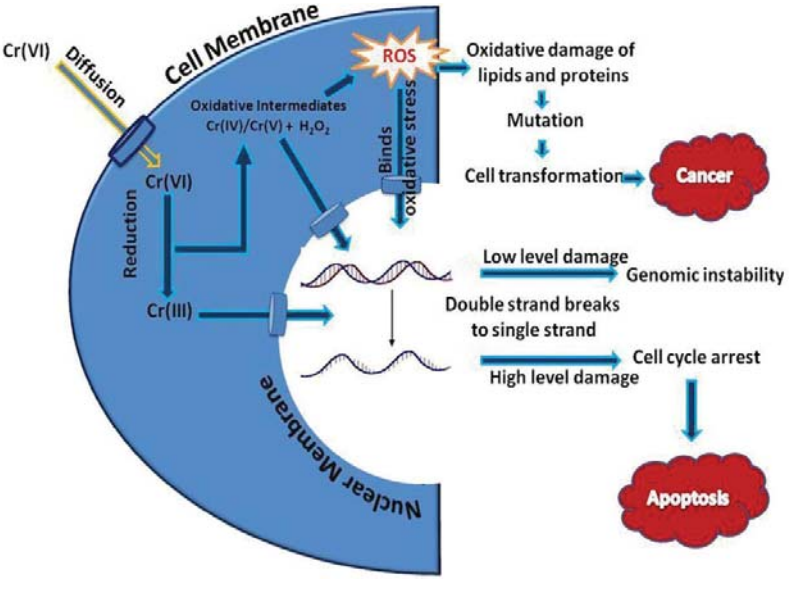

Figure 1. Schematic representation of cellular uptake of hexavalent chromium in red blood cells (Modified after Baduel. 2013. Figure 1. Schematic representation of cellular uptake of hexavalent chromium in red blood cells (Reprinted from Das & Singh, 2011).

Absorption and conversion of hexavalent chromium in blood

Cr (VI) is unstable in the body and is reduced intracellularly (by many substances including ascorbate and glutathione) providing very unstable pentavalent chromium and stable trivalent chromium. The erythrocyte has a high capacity for chromium (VI) uptake and binding. Cr (VI) enters the erythrocyte (Figure 1) through a sulfate ion channel; once inside the cell, it is rapidly reduced to reactive intermediates Cr (V) and Cr (IV)) and binds to the beta chain of human hemoglobin

(Kerger *et al.,* 1997) and other ligands like proteins and glutathione. The chromium-hemoglobin complex is stable and remains sequestered within the cell over the lifespan of the erythrocyte (Paustenbach *et al.*, 2003).

Both these intermediates can change DNA (Baduel, 2013). RBC membranes are however relatively impermeable to cationic trivalent chromium and when varying amounts of radioactive Cr (III) were added to whole blood *in vitro*, almost all of the radioactivity (94–99%) remained in the plasma with an insignificant count retained in the RBC. Similar results were obtained *in vivo* (Afolaranmi *et al.*, 2013).

Ingested Cr (VI) is efficiently reduced to Cr (III) by the gastric juices (De Flora, *et al.,* 1987) and ascorbate (Samitz, 1970) plays an important role (ATSDR , 2000) in this conversion. Inhaled Cr (VI) can be reduced to Cr (III) in the epithelial lining fluid of the lungs by ascorbate and glutathione (Petrilli *et al.,* 1986; Suzuki & Fukuda, 1990). According to Standeven & Wetterhahn (1989), Cr (VI) readily enters cells by diffusion through a nonspecific anion channel, whereas to Cr (III) the cells are relatively impermeable.

Thus if the amount of ingested Cr (VI) is higher than the reductive capacity of the gastrointestinal tract, both plasma and RBC chromium levels may be elevated for a few hours after ingestion, while RBC chromium levels may remain elevated for several weeks. In contrast, Cr (III) does not readily cross red blood cell membranes but binds directly to transferrin, an iron-transporting protein in the plasma (EPA, 1998; ATSDR, 2000; Dayan & Paine, 2001).

The cellular uptake of chromium was documented (Merritt and Brown, 1995) in red blood cells following corrosion of stainless-steel and cobalt-chromium implants *in vivo*, in red blood cells of patients undergoing total joint revisions and in fibroblasts, resulting in the release of the biologically active hexavalent chromium into the body. By applying the amberlite separation technique, Meriitt and Brown (1995) demonstrated that the hexavalent Cr in red blood cells was rapidly reduced to trivalent Cr. It was found that the excess Cr (VI) after being detoxified in saliva and sequestered by intestinal bacteria, if absorbed in the intestine, was efficiently reduced to its trivalent form and the efficient uptake and reduction of chromium(VI) in red blood cells reduced the chance of induction of cancer (De Flora, 2000).

Intra-tracheal instillation of $^{51}CrCl_3$ in anesthetized rabbits resulted in its partial absorption in blood and confinement of the absorbed material in the plasma compartment and only trace amounts were deposited in liver and kidney. By contrast, after similar application of Na, $^{51}CrO_4$ the bulk of blood radioactivity was detected from red blood cells (RBC) with substantial deposition in liver and kidneys, leading to the conclusion that Cr(VI), if it enters the body unreduced through the lung, will be partially deposited in cells over a prolonged period of time (Wiegand *et al.,* 1987).

By monitoring the reaction of Cr (VI) with GSH *in vitro,* it was found that GSH reduced Cr (VI) without any cofactors. GSH was also found to facilitate Cr(VI) uptake by reducing Cr(VI) to Cr(III) after entering the cell, presumably keeping intracellular Cr(VI) concentration low and allowing for further Cr(VI) uptake (Standeven and Wetterhahn , 1989). Some other nonenzymatic factors, like ascorbate and riboflavin, cytochrome P-450, DT-diaphorase and the mitochondrial electron transport chain complexes, are capable of reducing Cr (VI) *in vitro* but their contribution *in vivo* is not clear.

Biological monitoring for hexavalent chromium

The most reliable and direct way to measure the extent and the effect of hexavalent chromium exposure is to measure the Cr (VI) content of the RBCs and plasma, where chromium RBC levels provide information regarding exposure to Cr(VI) and chromium plasma levels can be useful for evaluating recent exposure to Cr(III) and Cr(VI) compounds. The concentration of chromium within the red cell depends upon inherent genetic polymorphisms (NIOSH, 2002).

Cr(VI) ions, taken by inhalation or percutaneously, are carried in blood plasma and usually penetrate into the erythrocytes depending on the concentration. The erythrocytes become an easily accessible target organ for quantitative chromium determination after occupational exposure to Cr(VI) compounds for intracellular reduction to Cr(III) and the concurrent intracellular protein binding.

Although the blood chromium levels (both plasma and RBC) for any individual may vary widely throughout the day due to fluctuations in dietary chromium intake and non-occupational exposures to chromium, experimental results (Buynder, 2010) showed no statistically significant differences between the mean blood erythrocyte or plasma chromium levels between the normal population and case groups with chromium intake from the diet or other non-occupational exposures to chromium.

Ramzan *et al. (*2011) concluded that chromium exposure in tannery workers led to low values of total erythrocyte count (TEC), packed cell volume (PCV) and mean corpuscular hemoglobin (MCH) in age groups between 20–60 years. They consider the hematological profile a potent indicator of chromium toxicity.

Huang *et al.* (1999) found the concentrations of both chromium and malondialdehyde (MDA), the product of lipid peroxidation in blood and urine, to be significantly higher in chromium-exposed workers from a chrome-plating factory. Their data suggest that MDA may be used as a biomarker for occupational chromium exposure but antioxidant enzymatic activities are not a suitable marker for chromium exposure.

Poznyak *et al.* (2002) found that exposure to Cr (VI) induced lipid oxidation, which again can be detected by ozonation and quantitatively calculated by the DB-index and DB_{cell}-index determination in plasma, erythrocytes, and sperm, and hence the ozonation method can be considered an acceptable modern technique (fast, inexpensive and simple) for chromium toxic effect monitoring *in vitro*.

A new type of biological monitoring method to find whether threshold concentrations have been respected over a given period was introduced by Lewalter, *et al.* (1985). On the other hand, the formation of DNA-protein crosslinks (DPCs) in target tissues appears to be the direct and primary genotoxic effect of Cr(VI) exposure and the lymphocytic DPCs may be viewed as a biomarker of internal Cr (VI) accumulation (Xiao *et al.,* 2013).

The possibility is however to be pointed out that small, exposure-related changes in hematological parameters may not have been detected in occupational exposure studies if values were within normal clinical ranges.

Hematological profiles

Cases of hematological changes have been reported in humans after ingestion of lethal or sublethal doses of Cr (VI) compounds. The blood report of an eighteen- year-old woman who ingested a few grams of potassium dichromate indicated decreased hemoglobin content and hematocrit, increased total white blood cell counts, reticulocyte counts, and plasma hemoglobin after four days of ingestion, clearly depicting intravascular hemolysis (Sharma *et al.*, 1978). Laboratory analysis of the blood sample of a 35-year-old woman who died 12 hours after ingesting 50 ml of pure chromic acid [25 g Cr (VI)] revealed severe anemia with thrombocytopenia. (Loubières *et al.*, 1999).

In a study of the National Toxicological Programme in 2008, male F344/N rats (6–7 weeks old) were exposed to sodium dichromate dihydrate in drinking water and hematological assessments were conducted after 22 days, 3 months, 6 months, and 1 year. The results indicated microcytic, hypochromic anemia in the test animals.

According to De Flora *et al.* (2000), the sequestering capacity of whole blood (187–234 mg per individual) and the reducing capacity of red blood cells (at least 93–128 mg) explain why this metal is not a systemic toxicant, except at very high doses.

Effects of Cr (VI) on plasma

Since RBC and plasma chromium are assumed to be in the hexavalent and trivalent form respectively, it appeared that there was some reduction of the hexavalent form in both fasted and nonfasted animals after a single oral dose (MacKenzie *et al.*, 1959). Spontaneous plasma reduction capacity (SPRC) determines the ability of an individual *to* reduce Cr (Vl) to Cr (lll) and once converted to trivalent form, chromium can no longer enter the red blood cell. Subjects with "strong" SPRC will thus excrete a relatively higher concentration of chromium in urine, leaving lower concentrations of chromium within the red blood cells (Miksche and Lewalter, 1997; World Health Organization, 1996; Geller, 20011).

In the absence of known exposure, whole blood chromium concentrations are in the range of 2–3 μg/100 mL, with lower levels occurring in rural areas only. It is therefore possible to distinguish sources and types of exposure [Cr (VI) versus other forms of Cr] by measuring Cr contents of RBC and serum (ASTDR, 2008).

In general, the reference values for chromium in plasma for populations that are not occupationally exposed to chromium range from 0.04–0.35 μg/L (Christensen *et al.*, 1993) with a mean of 0.25 μg/L. The average chromium concentration in plasma of residents was found to be 5.71 nmol/L or 0.27 μg/L (Torra *et al.*, 1999).

Pharmacokinetic patterns showed that Cr (VI) had the highest bioavailability (6.9%) and the longest half-life (approximately 39 hr) compared to its other forms and although all of them could cause temporary elevations in red blood cell (RBC) and plasma chromium concentrations. The highest efficacy was shown by Cr(VI) . By comparing RBC and plasma chromium patterns in animals exposed to high doses of Cr (VI) (Kerger *et al.*, 1996), it was found that nearly all the ingested Cr (VI) was reduced to Cr (III) before entering the bloodstream.

Effects of Cr (VI) on erythrocytes

When Cr VI was inhaled or administered intratracheally, intraperitoneally, or intravenously, much of the chromium in the blood (25 to 70%) was taken up by RBCs (Sayato *et al.*, 1980; Weber, 1983; Wiegand *et al.*, 1984; Edel & Sabbioni, 1985; Minoia & Cavalleri, 1988; Gao *et al.*, 1993). As the erythrocyte to plasma ratio of total chromium increases with increasing hexavalent chromium concentration, Corbett *et al.* (1998) proposed that the reductive capacity of erythrocytes was much greater than that of plasma and that the reduction rate of hexavalent chromium in erythrocytes was greater than the rate of uptake from plasma.

As discussed earlier, Cr (VI) taken up by RBCs undergoes reduction to the trivalent form with the help of reduced glutathione (Wiegand *et al.*, 1984) and complexes with Hgb and other intracellular proteins that are sufficiently stable to retain chromium for a substantial fraction of the RBC lifetime (Aaseth *et al.*, 1982). This was confirmed by the result of an experiment where $K_2Cr_2O_7$, a hexavalent chromium compound, introduced into plasma and reconstituted whole blood from three individuals was found to be readily reduced to Cr (III) in the concentration range of 100–1,000 μg Cr (VI)/L (Corbett *et al.*, 1998). Excess trivalent chromium in the RBC is sequestered until cell death (Kerger *et al.*, 1997; Aaseth *et al.*, 1982). Over time, the RBC-associated chromium appears to be transferred to the spleen as a result of scavenging aging RBCs from the blood.

The total chromium content in the blood of workers of age group 1–20 years was significantly higher (24%) when compared with the same age group of non-workers. The mean cell volume, packed cell volume and platelet counts in workers were generally lower, whereas the hemoglobin, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration values were higher in workers than in non-workers. Total erythrocyte count (TEC) was

found to be significantly lower in tannery workers of age group 20–50 years, regularly exposed to Cr (VII) than in their respective non-exposed control counterparts. Packed cell volume (PCV) was significantly higher in workers of age group 30–40 years, while mean corpuscular hemoglobin (MCH) was significantly lower in the age group above 50 years. No significant differences were observed in the values of hemoglobin, mean corpuscular volume and mean corpuscular hemoglobin concentration between tannery workers and non-exposed control individuals (Ramzan *et al.*, 2011), whereas the hemoglobin and mean corpuscular hemoglobin concentration values were higher in workers than in non-workers.

Beyersmann and Buttner (1989) detected modification of human erythrocyte membrane proteins by chromate and observed that chromate (10 mM) caused an increase in the intracellular resistance with an augmentation of the critical voltage, where the membrane resistance broke down owing to electroporation. Moreover, a slight chromate-induced augmentation of echinocyte shape was observed. Chromate also caused the intracellular pH to shift to higher values. Stana *et al.* (2009) observed increase of erythrocyte membrane fragility in direct relation with the administered dose of chromium. Significant differences between experimental and control groups were registered and the degree of hemolysis was related to the dose.

As the human plasma was found capable of spontaneous reduction of Cr (VI) ions of up to 2 ppm to Cr (III), it was assumed that only those Cr (VI) concentrations can penetrate the membrane of the RBC and enter the cell which either come into contact with the membrane during the reduction process or exceed the limit concentration of 2 ppm. This reduction capacity (PRC) can be increased considerably by adding ascorbic acid (AA) as decreased binding of Cr (VI) inside the erythrocytes has been reported under the effect of AA. Thus a sizable portion of the increase of chromium levels in plasma and RBC following oral administration of Cr (VI) to humans is probably due to the accumulation of Cr (III) formed by the extensive reduction of absorbed Cr (VI) in plasma and RBC after oral administration (OEHHA, 2011)Nejla Soudani *et al. (*2011) found excess chromium (Cr) exposure to be associated with various pathological conditions including hematological dysfunction. The generation of oxidative stress is one of the plausible mechanisms behind Cr-induced cellular deteriorations. The efficacy of selenium (Se) to combat Cr-induced oxidative damage in the erythrocytes of adult rats was investigated by studying the effects of Se by providing selenium enriched and selenium-free hexavalent chromium mixed diet with drinking water to female Wistar rats for 3 weeks, maintaining proper controls. The rats exposed to only hexavalent chromium showed an increase of malondialdehyde and protein carbonyl levels and a decrease in sulfhydryl content, glutathione, non-protein thiol, and vitamin C levels. A decrease of enzyme activities like catalase, glutathione peroxidase, and superoxide dismutase activities was also noted, whereas co-administration of selenium could restore the given parameters to near-normal values to prevent Cr (VI)-induced erythrocyte damage.

Exposure to high chromium values in the gestation period led to increased chromium level in mother blood, placenta and fetus, with significant decrease of hemoglobin ($p<0.01$) in experimental groups compared to controls (Stana *et al.*, 2009)

Experiments of Alpoim *et al.* (1995) and Fernandes *et al.* (1999) pointed out the occurrence of chromate-induced hemoglobin oxidation and membrane peroxidation in human erythrocytes, which in turn promoted oxidation of GSH, inhibition of glutathione reductase and methemoglobin reductase, and the transformation of normal shape to echinocytic form of erythrocytes. However, chromate did not affect the activities of catalase, gluthatione peroxidase, superoxide dismutase and did not hamper the osmotic fragility of the cells (Fernandes *et al.*, 1999). Based on these findings, it was suggested that chromate may be cytotoxic to human erythrocytes. It should be taken into account that in human erythrocytes the intracellular redox balance, reduced glutathione oxidized, hemoglobin/ methemoglobin ratio, and the cell shape, which are crucial for cell functions and survival, are irreversibly disrupted by Cr (VI) (Fernandes *et al.*, 1999). The finding showing that pretreatments of human erythrocytes with vitamin E, vitamin C, salicylate and deferoxamine (DFO) potentiated chromate-induced cytotoxicity, indicating that pre-treatment of cells with DFO prevented chromate-induced peroxidation, revealed that these drugs potentiated the electron transfer between the hemoglobin-Fe^{2-} and chromium (V) intermediates, decreasing chromium (V) intermediates-mediated generation of ROS via the Haber-Weiss cycle or through a Fenton-like reaction (Fernandes *et al.*, 2000).

Results of acute, intermediate, and chronic treatment studies in animals identified that the hematological system is one of the most sensitive targets of oral exposure to chromium (VI) and the effects include microcytic, hypochromic anemia, characterized by decreased mean cell volume (MCV), mean corpuscular hemoglobin (MCH), hematocrit (Hct), and hemoglobin (Hgb), as observed in rats and mice orally exposed to chromium (VI) compounds for 4 days to 1 year. The severity of anemia exhibited dose- and duration-dependence, with maximum effects observed after approximately 3 weeks of exposure; but with increasing exposure durations, anemia became less severe, presumably due to compensatory hematopoietic responses.

In Swiss mice, intraperitoneally injected hexavalent chromium, blood hemoglobin level, hematocrit value and erythrocyte count were reduced by 17.5, 17.4 and 15.9%, respectively, as compared to controls, accompanied by echinocytic transformation (Figure 2) of 33.8% erythrocytes, indicating hemolytic anemia. But cytochemical studies indicated that Cr (VI) treatment did not cause denaturation of already synthesized hemoglobin (Ray & Sarkar, 2012).

Results of hematological analyses showed that mice exposed to sodium dichromate dihydrate in drinking

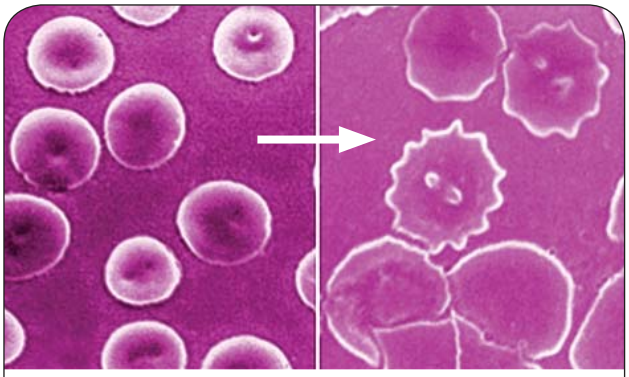

Figure 2. Echinocytic transformation of hexavalent chromium exposed normal mammalian erythrocytes.

water for 3 months developed mild erythrocyte microcytosis (Stern & Dabt, 2009. The effects were however more severe in rats exposed under similar conditions.

Although after treatment of ≥5.2 mg hexavalent chromium/kg-day, MCV and MCH were significantly reduced in males (maximum 8%) and females (maximum 10%), erythrocyte counts were slightly increased in females, but not in males (Bucher, 2007).

Acute exposure of male rats to 2.7 mg chromium (VI)/kg/day in drinking water for 4 days, produced a statistically significant decrease (2.1%) in MCH. Further increase in the dose (≥7.4 mg chromium (VI)/kg/day) resulted in additional decrease in MCH and decrease in MCV. Although the magnitude of changes of hematological parameters after acute exposure was minimal, still these are considered to be indicative of adverse health effects.

More severe microcytic, hypochromic anemia occurred in rats and mice following exposure to sodium dichromate dihydrate in drinking water for 22 or 23 days, which was found to ameliorate with time (NTP, 2008). Decreased Hct, Hgb, MCV, and MCH occurred at ≥0.77 mg chromium (VI)/kg/day, with decreases exhibiting dose-dependence; effects were not observed at 0.21 mg chromium (VI)/kg/day, but after exposure for 3–12 months, severity of the microcytic, hypochromic anemia in treated rats and mice was reduced. Hematological effects, including decreased hematocrit, hemoglobin, and erythrocyte count, have also been reported in rats exposed to chromium trivalent oxide for 90 days. A dose-dependent reduction of the peripheral blood erythrocyte count and a decrease in hemoglobin level were observed in experimental animals after intraperitoneal injections of Cr (VI) in the concentrations of 0.025 μg/kg to rats (Zhumabaeva *et al.,* 2014).

Stana *et al.* (2009) evaluated hexavalent chromium toxicity in Wistar female rats, which received "*in utero*"during the gestation period different doses of Cr (VI), namely 25, 50 and 75 ppm. They found precipitation of hemoglobin, appearance of Heinz body, reduction of plasticity followed by irreversible self-oxidation of saturated lipids, and finally degeneration and lysis of erythrocytic membrane.

Richelmi *et al.* (1984) immediately analyzed the hematological parameters of blood collected at different times, after intravenous administration of 0.5 and 2.5 mg/kg b.w. of $K_2Cr_2O_7$ in male Wistar rats. Cr (VI) was not detected in whole blood one minute after administration of the lower dose. In blood of rats receiving the higher dose, an incomplete reduction of Cr (VI) was observed, revealing a highly rapid but limited metabolic capacity of hematic compartment to reduce Cr (VI) to Cr (III).

Subcutaneous introduction of a lower dose (10 mg/l) of Cr (VI) induced a marked decrease in the number of erythrocytes (–6%), hematocrit values (–15%) and hemoglobin concentration in male Wistar albino rats, although a comparatively higher dose (30 mg/l) in drinking water had no effect on the erythropoietic parameters studied. Short-term subcutaneous introduction of lower dose exposures to female Wistar albino rats resulted in eythrocytopenia and a decrease in hematocrit values and hemoglobin concentration (Adjroud *et al.*, 2009).

"*In vitro*" incubation of $K_2Cr_2O_7$ (4 μM) with rat erythrocytes or plasma at 37°C showed a rapid reduction of Cr(VI) in red cell while plasma samples demonstrating a limited reductive power along with a decrease in hematocrit, particularly due to the change in blood fluid volume (Stern *et al*, 2009).

Effects of Cr (VI) on leukocytes

The white blood cell counts decreased in workers up to the age of 40 years, whereas in the older population it showed a slight increase (Ashan *et al.*, 2006). The transport characteristics of labelled chromium (51-chromate) in normal human leukocytes indicates that chromate uptake is highly specific, unidirectional and follows the Michaelis-Menten kinetics (the maximum velocity is 52 m/moles/g dry weight of cells per min). Further it is temperature sensitive and energy dependent. A variety of metabolic poisons, including metavanadate, competitively inhibits chromate influx. On the basis of their experimental results, Lilien *et al.* (1970) proposed that Cr (VI) may be the form in which chromium penetrates the cell membrane, operating on a highly specific transport mechanism.

After intraperitoneal injection with an aqueous solution of potassium dichromate at a dose (one tenth of LD_{50} value) for 5 consecutive days per week, for a total period of 2 weeks in male albino Swiss mice, it was found that although there was no change in the TC and DC of leukocytes at the initial stage, at the end of the second week of treatment, TC of leukocytes was noted to be markedly reduced in the experimental mice, as compared to that in the controls. Interestingly, the count of lymphocytes was considerably higher in the experimental mice than in their control counterparts, while the count of neutrophils was significantly lower. These findings along with a higher incidence of chromosomal abnormalities and micronucleated cells in the bone marrow made the authors (Ray & Sarkar, 2011) conclude that hexavalent

chromium is relatively more myelosuppressive than lymphopenic in action. Microscopically lymphoid hyperplasia was characterized by minimal-to-mild lymphocyte proliferation. In adult Swiss mice, treated intraperitoneally with hexavalent chromium, Ray and Sarkar (2012) found leukopenia only after 2 weeks (mean leukocyte count: 4.91 thousand c mm^{-1}). Subcutaneous administration of hexavalent chromium (50 mg/Kg body weight) in male Wistar albino rats, led to leukopenia (–55%), lymphopenia (–57%), monocytosis (+104%), and granulocytosis (+204%). Subcutaneous exposure to a low dose of $K_2Cr_2O_7$ in female Wistar albino rats caused almost similar changes in the leukocytic profile. Oral administration of Cr (VI) through drinking water seriously affected male rats by inducing leukopenia, lymphopenia, monocytosis, and granulocytosis (Adjroud, 2009).

Lei and Zhuang (1995) found the formation of DNA-protein cross links (DPC) in many tissues in male Sprague-Dawley (SD) rats, following repeated intraperitoneal injection of Cr (VI) (10 mg/kg) for 3 weeks, with WBC found a highly sensitive target of chromate(VI).

The study of Patlolla *et al.* (2009) demonstrated that intraperitoneal administration of Cr (VI) to rats during 5 days could induce DNA damage in peripheral blood lymphocytes and oxidative stress in liver and kidney.

Krupa *et al.* (2002) exhibited the role of Cr (VI) on RNA and DNA-chromium adduct formation in isolated nucleic acids and isolated pig lymphocytes. The incubation of total cellular RNA and nuclear DNA isolated from lymphocytes with Cr (III) and Cr (VI) yielded a binding of Cr atoms to RNA 1.1D1.6 higher than to DNA and the number of chromium atoms bound to nucleic acids was higher after incubation with Cr (VI) than with Cr (III) in both experimental systems.

Halasova *et al.* (2012) proposed that although no apparent increase in chromosomal damage was recorded in chromium-exposed welders in comparison with controls, the genetic make-up in DNA repair genes may increase the susceptibility toward adverse effects of chromium. Hexavalent chromium was found to induce DNA-protein crosslinks (DPCs) and mitochondrial damage to lymphocytes and the lymphocytic DPCs may be viewed as a biomarker of internal Cr (VI) accumulation (Xiao *et al.*, 2013).

Pilot studies of DNA-protein cross-links in peripheral blood lymphocytes have been conducted by Costa *et al.* (1996) in individuals with higher exposure to chromate (welders) and individuals with lower levels of exposure (residents living in a chromium-contaminated area) in New Jersey and in two Bulgarian cities (Jambol and Burgas) with different levels of air pollution and Cr(VI) exposure. DNA protein cross-links in welders and in individuals living in New Jersey around chromium-contaminated areas were significantly higher compared to matched controls.

A small group of 5 manual metal arc stainless steel welders exposed to hexavalent chromium were examined for two end-points: a chemical one with the formation of DNA-protein crosslink (DPC) and a biological one marked by the occurrence of micronuclei in peripheral lymphocytes by Medeiros *et al.* (2003). Zhang *et al.* (2011) found that low-level occupational exposure to hexavalent chromium induced DNA damage in peripheral lymphocytes in electroplating workers. Werfel *et al.* (1998) measured DNA damage and sister chromatid exchange (SCE) frequencies in lymphocytes taken from the venous blood of an equal number of welders and non-welders, with the welders showing a significantly higher rate of DNA single-strand breakages and significantly elevated SCE values. Moreover, DNA single-strand breakage and DNA-protein cross-links differentially increased depending on the exposure levels to chromium (VI).

Effect of Cr (VI) on lymphocytes

In experimental animals, Zhumabaeva *et al.* (2014) found on the 30th day of treatment peripheral blood lymphocytes and leukocytosis developed at the expense of higher counts of B (CD_{20}) and T lymphocytes (CD_3) and their subpopulations. Intraperitoneal injections of Cr (VI) brought about a significant change in the morphology of the thymus gland and increased the counts of macrophages. *In vitro* experiments on murine lymphocytes also led to inhibition of the proliferation of both T and B cells. This immunosuppression was associated with the development of implant-associated infection in patients with a prosthesis (Wang *et al.*, 1997). Phagocytic activity of alveolar macrophages and the humoral immune response were depressed in the presence of higher doses of Cr (VI) (Glaser *et al.*, 1985). Terpilowska and Siwicki (2010) showed that chromium injection (dose of 1 and 10 mg Cr per body weight) significantly decreased IL-1α concentration but not the concentration of IL-6 and induced no differences in the proliferative response of lymphocytes and in the metabolic activity of phagocyting cells.

Studies of Quievryn *et al.* (2001) showed that reduction of Cr (VI) by cysteine resulted in the formation of mutagenic Cr (III)-DNA adducts in the absence of oxidative DNA damage. They found that the peripheral lymphocytes from unexposed humans had a 7.8-fold excess of glutathione over cysteine, whereas lymphocytes from stainless steel welders contained only a 3 times higher amount of glutathione, which was entirely caused by the decrease in the concentration of glutathione. The higher reduction rate combined with a decrease in the intracellular concentration of glutathione made cysteine a predominant Cr (VI)-reducing thiol in lymphocytes of welders.

Effect of Cr (VI) on platelets

Royer *et al.* (2010) found that after taking oral chromium picolinate tablets for 4–5 months for losing weight, the patient developed acute thrombocytopenia. Platelet count and other abnormalities returned to normal by day 26 after stopping chromium tablets. Subcutaneous exposure (50 mg/kg body weight) of Cr (VI) in male Wistar albino rats, which after 3 days led to thrombocytosis (+38%), resulted in the long run in a marked decrease in the number of platelets (–48%). Short-term subcutaneous

exposure to low doses of $K_2Cr_2O_7$ induced thrombocytopenia in female Wistar albino rats. Oral treatment of 30mg/l Cr (VI) with drinking water to male Wistar albino rats reduced the platelet count during the first three days, while on the 6th day of chromium treatment the situation was reversed with an elevation (+21%) in platelet counts.

Effect of Cr (VI) on induction of apoptosis and cancer

Vasant *et al.* (2001) reported that apoptosis was the mode of cell death of human lymphocytes in the presence of both Cr (V) and Cr (VI). In Fanconi anemia (FA), an autosomal recessive disorder in humans, the chromium DNA crosslinking might act as proapoptotic lesion. Bagchi *et al. (*2001) demonstrated concentration- and time-dependent effects of Cr (VI) on DNA fragmentation and apoptotic cell death in human peripheral blood mononuclear cells.

NIOSH considers all Cr (VI) compounds to be occupational carcinogens and recommends that airborne exposure to all Cr(VI) compounds be limited to a concentration of 0.2 μg Cr(VI)/m^3 for an 8-hr time-weighted average exposure, during a 40-hr workweek (occupational exposure). Cr(VI) is implicated as respiratory carcinogen inducing several types of DNA lesions, including ternary DNA-Cr-DNA interstrand cross-links (Cr-DDC) (Vilcheck *et al.*, 2002). Hexavalent chromium is a known human carcinogen via inhalation (IARC, 2012; OSHA, 2006; U.S. EPA, 1998a), though less is understood about the risks of hexavalent chromium when ingested (Stern, 2010). Observational epidemiology studies of a population in China reported conflicting results on whether an association exists between consumption of drinking water contaminated with hexavalent chromium and stomach cancer (Kerger *et al.*, 2009; Beaumont *et al.*, 2008; Zhang & Li, 1997). On the other hand, absorption of Cr (VI) into the intestinal epithelium, oxidative stress and inflammation, cell proliferation, direct and/or indirect DNA modification, and mutagenesis (Thompson *et al.*, 2011) are key events of tumorogenesis.

Biochemical profiles

The total chromium (56.9%) and hexavalent chromium (78%) were significantly higher in the blood of an exposed male and female worker population as compared with the control population. No definite pattern was observed in different hematological and biochemical parameters when comparing workers with non-workers, normal males with exposed males and normal females with exposed females. A slight variation may be due to a multitude of factors in addition to possible effects of chromium toxicity (Ahsan *et al.*, 2006).

Albumin, alkaline phosphatase, alanine aminotransferases, aspartate aminotransferases and total protein showed higher values, whereas the total bilirubin, direct bilirubin and blood serum glucose contents showed lower values in blood sera of factory workers, both male and female, when compared with those of the control population.

EL-Shafei (2012) found that there was a significant elevation in the level of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), creatinephosphokinase (CPK) in blood samples of workers of chromium-electroplating factories.

Cr (VI) was found to decrease superoxide dismutase (SOD) and reduced glutathione (GSH), to increase glutahione peroxidase (GPx) in human erythrocytes and to reduce the ferric reducing ability of plasma (FRAP) (Dlugosz *et al.*, 2012).

Remedial measures of adverse hematological effects of Cr (VI)

Industrialization, urbanization and various anthropogenic activities such as mining and agriculture have increased releases of toxic heavy metals into the natural environment, altering both natural and man–made ecosystems (Mudhoo *et al.*, 2012). Excess chromium (VI) exposure is associated with various pathological conditions including hematological abnormalities. The generation of oxidative stress is one of the plausible mechanisms behind Cr-induced cellular deteriorations. The efficacy of selenium (Se) to combat Cr-induced oxidative damage in erythrocytes of adult rats was investigated by Soudani *et al.* (2011). Interestingly, chromium hexavalent is extremely reactive with vitamin C. As Cr (VI) exposure was coupled with vitamin C in the body, Anatoly Zhitkovich and his team in 2007 found that vitamin C inside cells provoked more mutations and DNA breaks, turning chromium more toxic (Reynolds *et al.*, 2007). On the other hand, Xiao *et al.*, 2013, using peripheral blood lymphocytes from Sprague-Dawley rats, demonstrated that pre- and co-treatment with vitamin C had a protective effect against Cr (VI)-induced loss of cell viability and mitochondrial damage, whereas only vitamin C co-treatment had a protective effect against the Cr(VI)-induced increase in DNA-protein cross links (DPCs) correlated with expression of p53. However, vitamin C may only be effective in increasing elimination of Cr (VI) at high concentrations when plasma reduction is saturated and may be of limited therapeutic use in patients with orthopedic implants (Afolaranmi and Grant, 2013). The work of Rudrama Devi and Naik (2011) showed that the genotoxic effect associated with occupational exposure to high chromium levels could be significantly reduced by three months by ascorbic acid supplementation and the industry management was advised to use vitamin C in workers continuously inhaling fumes of Cr (VI). Tarasub *et al.* (2008) reported that the antioxidant quercetin, a potent oxygen free radical scavenger and a metal chelator found in fruits and vegetables, might have a protective effect against hexavalent chromium induced chromosome aberrations in rat bone marrow (Tarasub *et al.*, 2008).

Corbett *et al. (*1998) reported data indicating that the plasma reduction capacity is enhanced by a recent meal, yet it may be overwhelmed at Cr (VI) concentrations between 2000 and 10000 micrograms/L.

Luczak and Zhitkovich (2013) found a broad spectrum of chemoprotective roles of the antioxidant N-acetylcysteine (NAC) in human cells, including suppression of cytotoxicity, apoptosis, p53 activation, and HSP72 and HIF-1α upregulation. Cytoprotection by NAC was independent of cellular glutathione. NAC strongly inhibited the uptake of Cr (VI) causing a loss of Cr (VI) accumulation by cells.

Conclusions

On balance then, the major conclusion is that Cr (VI) induced toxicity or carcinogenicity is the consequence of altered cytogenomics associated with oxidative stress, DNA damage, apoptosis, cell-cycle regulation, cytoskeleton, morphological changes, energy metabolism, biosynthesis, oncogenes, bioenergetics, and an immune system critical for toxicity (Nigam *et al.,* 2014). The intensity of dysregulation of genes or pathways involved in mechanistic events forms a sub-threshold or threshold level depending upon the dose of the toxicant, duration of exposure, type of target cells, and niche microenvironment of cells resulting in several abnormal features. Blood cells in the body, an easy target to be affected by Cr (VI), produce alterations in normal hematological parameters leading to a number of ailments.

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