CHARACTERIZATION OF DNA-PROTEIN COMPLEXES INDUCED BY CR (VI) IN LIVER TISSUE OF RATS

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ABSTRACT

Chromium (VI) compounds have been found to produce carcinogenic and toxic effects in animals. In rats, chromium (VI) caused more damage in liver after an i.p. administration of K₂CrO₄. The liver tissue accumulated with K₂CrO₄ and make to protein bound to DNA, nuclear protein complexes were formed in liver after treated with K₂CrO₄. DNA crosslinks were found in nuclei isolated from the liver of rats treated with K₂CrO₄. Liver nuclei contained Protein associated DNA single strand breaks in addition to DNA-Protein cross links (DPCs) complexes isolated from both the control and K₂CrO₄ treated cells were analyzed by gel electrophoresis. The band appeared after treatment of potassium chromate indicates that 65 kDa acidic proteins crosslinked to DNA. The agarose gel electrophoresis shown the presence of average size of DNA was approximately 24000 to 750 base pairs as compared with standard marker. Chromium (VI) has been classified as an initiator of carcinogenesis on the basis of its genetic toxicity.

INTRODUCTION

Chromium (Cr) is ubiquitous in the environment, occurring naturally in soils, rocks, and living organisms. Cr exists in primarily two valence states, Trivalent Cr (III) and hexavalent Cr (VI), with the latter primarily produced by anthropogenic sources. Cr (III) and Cr (VI) are produced by many different industries including welding, chrome plating, chrome pigmenting, the ferrochrome industry and leather tanning (Fishbein, 1981). It is estimated that several hundred thousand workers are potentially exposed to high level of chromium (VI) (IARC 1990).

Hexavalent chromium compounds Cr (VI) are recognized human and animal carcinogen (Mattaagajasingh et al. 1996). Chromium (VI) compound administered to animal produced tumors but chromium (III) compounds do not produce them (ERA 1984). Mutagenicity of chromium (VI), but not chromium (III) has been well documented in various bacterial assay systems (Langard 1990). Interestingly, Cr (VI) does not bind to DNA or proteins in cell free system (Lay and Levina, 1998). However, Cr (VI), which exists as an oxyanion at physiological pH, is readily transported into the cell through the cell’s sulfate anion transport system. Inside the cell Cr (VI) is believed to be reduced by the cell’s redox system to its biologically most stable form, chromium (III) (Kitagawa, 1988). Cr (III) binds to DNA as well as proteins in cell free system and has high affinity for many other biological ligands. Cr (III), However is poorly taken up into the cell and is considered to be noncarcinogenic. (Linderg and Vesterberg, 1983). During the intracellular re-
duction of Cr (VI) to Cr (III), reductive species such as intermediate valence states of chromium and active oxygen species are generated. Which may in turn, initiate the carcinogenic process by altering the structure of DNA. Although chromate-induced DNA-protein complexes are implicated in chromate carcinogenicity, the mechanism of their formation, composition, and biological significance are not well understood. Therefore, it is necessary to know the identity of the protein that participate in chromate-induced DNA-Protein complexes and the nature of their interaction with DNA. In the present study, we have analyzed the proteins complexed to DNA by chromate treatment of animal model. The composition and stability of chromate induced DNA-Protein complexes and the effect of antioxidants on the formation of such complexes have also been reported here.

MATERIALS AND METHODS

Animal study

Male Wistar rats 100-120g were held for 5 days before experiment. Animals were divided into three groups and injected as follows: 1. Control normal saline 1ml/Kg body weight, 2. K2CrO4 was injected at a dose of 10 u mol/Kg and 3. K2 CrO4 at 25 u mol/Kg of body weight by intraperitoneally. The rats in all groups were sacrificed 10 days post treatment. The liver was removed and washed with saline to maintain under identical environmental conditions.

Isolation of DNA-Protein complexes

The method used to isolate DNA-protein complexes was modified previously described by Mattagajasingh & Misra (1995). The rats were sacrificed and liver removed after 10 days of experiments. Potassium chromate treated and control animal liver cells were collected by centrifugation at 10,000 rpm for 10 min and were washed three times in phosphate buffered saline. The cell were lysed in 10 ml of 10 mM Tris containing 2% SDS, 1mM HEPES/PMFS (pH 7.5) and were rinsed in saline. The pellets were centrifuged at 10,000 rpm for 10 min at 4°C, using refrigerates centrifuge. The gel run was carried out at 20v 120min, gel was stained with ethidium bromide(1ug/ ml) and the bands were visualized under UV illumination and the results were photographed (Sambrook et al. 1989).

RESULTS AND DISCUSSION

The LD50 determination results of potassium chromate for intraperitoneal route indicated that 50% mortality was observed at 60µmol of potassium chromate. (As shown in Fig.1) Therefore 10, and 25 µmol concentration of potassium chromate were used for the analysis; there was increased complexes formation on the basis of dose dependence. These resulted in dose dependent increases in the formation of DNA-protein complexes in liver tissues. When ever increasing the concentration of K2CrO4 treatment resulted increasing DNA-protein complexes as compared with the control. K2CrO4 induced DNA-protein complexes formation in liver was also found to be time dependent a shown in Fig.2.
not affect cell viability but cross-linked the same proteins to DNA.

There is of course, a possibility that the cytoplasmic proteins might associate with DNA during the cell lysis. We analyzed DNA-Protein complexes isolated from either whole cells or purified intact nuclei of cells treated with K$_2$CrO$_4$. That was not the case illustrated by the fact that identical proteins were used as starting material. Hence, it appears likely that K$_2$CrO$_4$ induces the cross-linking of nuclear proteins to DNA.

As shown in this Figure 3, all of the proteins cross-linked to DNA by 25µmol K$_2$CrO$_4$ treatment and a 65 kDa acidic protein cross-linked to DNA by higher doses of K$_2$CrO$_4$ were found in this fraction. These results suggest that nuclear matrix proteins are the targets for chromate-induced DNA-Protein cross-linking. The chromate-induced DNA-protein cross-links, which were DNA-Protein in nature, effectively masked the single strands breaks. Liver nuclei from chromate-treated rats caused DNA elution rates to increase slightly compared to control.

Stability of DNA-Protein complexes/ DNA agarose gel

The stability of DNA-Protein complexes was tested by monitoring the recovery of DNA and protein in the following treatment of DNase I, RNase and proteinase K, B-mercaptoethanol. The average size of DNA was approximately 24500, 15000 and 750bp pairs bands observed by gel. (Fig.4). The control sample had almost 100% recovery of both DNA and protein in the pellet by centrifugation. A treatment of DNA protein complexes did not interfere with the recovery of DNA or protein. Proteinase k treatment dissociates most of protein from the DNA-Protein complexes without affecting the recovery of DNA. This data indicated that chromate treatment induce the cross-linking of protein to DNA and does not cause sedimentable protein aggregates for chromate induced DNA-Protein complexes in the rat liver cells.

In the present study, Chromium (VI) caused more damage in rat liver after an i.p administration of K$_2$CrO$_4$. The level of chromium accumulated in rat liver has been measured after 10 days, of treatment with K$_2$CrO$_4$. The liver tissue was accumulated large of chromium and concentrated in the nucleus. Chromium was bound to DNA, nuclear DNA-protein complexes were formed in liver. The significant amounts of chromium were bound to DNA and non histone proteins. Chromium enters the liver at faster way and increasing the level of chromium bound to liver nuclear protein was observed in the experimental samples. It is possible that Cr (VI) induces a cytoplasmic factors, which influences the uptake and subsequent binding to nuclear protein complexes. Chromium DNA adducts was formed after K$_2$CrO$_4$ on the oxidation state of the chromium compound administered. Our data suggested that certain type of Cr-DNA complexes form after administration of Cr (VI) not produce lesion on the DNA. Liver nuclei contained protein associated DNA single strand breaks in addition to DNA-protein cross-links. The DNA damage induced by chromate is discussed in relation to the carcinogenicity and toxicity of chromium (VI) compounds.

Chromium (VI) containing compounds are recognized occupational carcinogens and pose an environmental health risk. Chromium (VI) has been classified as an initiator of carcinogenesis on the basis of its genetic toxicity.

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REFERENCES


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LEGENDS
M - Commercially purchased marker
C - Control (Protein isolated from normal rat liver)
1 - 10 µm of K$_2$CrO$_4$
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