Study on the DNA-protein crosslinks induced by chromium (VI) in SPC-A1

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Abstract. Objective: This study was designed to investigate the effect of chromium (VI) on DNA-protein crosslinks (DPC) of SPC-A1 cells. Methods: We exposed SPC-A1 cells were cultured in 1640 medium and treated with the SPC-A1 cells in vitro to different concentrations of Hexavalent chromium Cr(VI) for 2h , the KC1-SDS precipitation assay were used to measure the DNA-protein cross-linking effect. Results: All the different concentrations of Cr(VI) could cause the increase of DPC coefficient in SPC-A1 cells. But this effect was not significant ($P>0.05$) at low concentrations; while in high concentration Cr(VI) induced SPC-A1 cells could produce DNA-protein cross-linking effect significantly ($P<0.05$). Conclusions: chromium (VI) could induce DNA-protein crosslink.

1. Introduction

Chromium, the seventh most plentiful element in the earth's crust (0.1–0.3 mg/g), is a redox active 3d transition metal from group VI-B of the periodic table with diverse oxidation states where in the trivalent Cr3+ species and hexavalent Cr6+ forms are ecologically significant, since these are the most stable oxidation states in the natural environment. [1] Whether chromium is really an essential element for organisms is questionable as a low concentration of chromium (Cr3+) in the diet displayed no toxicity in current studies and no essential metabolic role is known till date. [2]The toxicity of chromium is dependent on its oxidation state. Moreover, the United States Environmental Protection Agency (USEPA) has included Cr6+ among 17 chemicals which are considered the most hazardous to human health.Chromium (Cr) is one of the toxic heavy metals, Generally, Cr exists in two stable states: trivalent chromium [Cr(III)] and hexavalent chromium [Cr(VI)]. The toxicity of Cr for the different valences is quite different. Cr(III) is an essential trace element that plays an important role in glucose and cholesterol metabolism, but excessive intake of Cr(III) may bring about damage. Cr(VI) is generally considered 1,000 times more toxic than Cr(III).[3]

Chromium pollution is increasing incessantly due to continuing industrialization. The hexavalent chromium [Cr(VI)] is a common contaminant found in soil, air and water. Of various oxidation states, Cr6+ is very toxic due to its carcinogenic and mutagenic nature. It can be ingested via digestive tract, respiratory tract and skin contact, and directly or indirectly cause severe health problems in humans. [4]Hexavalent chromium (Cr(VI)) is known to induce lung cancer and is genotoxic in a number of in vitro systems causing DNA-strand breaks, DNA-DNA cross links, DNA-protein cross links and Cr-DNA adducts. [5]Many epidemiologic studies of Cr(VI)-exposed workers in industries located in

Europe/UK, the USA, Japan, and other countries focused on oral cancers, esophageal cancer, lung cancer, and gastrointestinal cancers and reported the risk estimates for highly exposed subcohorts.[6]

It has been reported that chromium (Cr(VI)) induces DNA-protein crosslinks in many cell lines. However, the morphological characteristics leading to DNA-protein crosslinks or subsequent regeneration in cells exposed to chromium (Cr(VI)) have not been reported in the SPC-A1 cells.so, we study In the experiment, different doses chromium (Cr(VI)) were treated chromium (Cr(VI)) poisoning. By detecting the change of hydroxyl radical inhibiting capacity and DPC content in SPC-A1 cells. The damage of chromium (Cr(VI)) on DNA of SPC-A1 cells. Was explored to provid the foundation for studying toxicity machanism of chromium (Cr(VI)).

2. Materials and methods

2.1. Materials

Potassium dichromate was obtained from Bodi Chemical Company in Tianjin, China. SDS and proteinase K were purchased from Merk Company. Hoechst 33258 fluorochrome and calf thymus DNA were bought from Sigma Company (Beijing, China). The other chemical reagents were all analytical pure reagents made in China. SPC-A1 cell was from our laboratory.

2.2. Cell culture and treatments

SPC-A1 cells were maintained in RPMI 1640 medium. The media were supplemented with 10% fetal bovine serum (FBS), 100 units penicillin/ml and 100μg streptomycin/ml, under standard conditions in a 37℃ incubator with a humidified mixture of 5% CO2 and 95% air. SPC-A1 cells treated with five doses of Potassium dichromate: (0μmol/L, 2.5μmol/L, 10μmol/L, 40μmol/L, 160μmol/L) and control. The control group was treated with PBS. After 2h, five doses of Potassium dichromate were put in 1.5 mL centrifuge tube the KC1-SDS precipitation assay were used to measure the DNA-protein cross-linking effect.

2.3. Measurements of DNA –protein crosslinks (DPC) [7-8]

The number of DNA – protein crosslinks (DPC) was determined by a K-SDS precipitation assay, as modified. SDS can bind to DPC and other protein, but not to the free DNA. When KC1 was added to samples, DPC and protein precipitated while the free DNA remained in the supernatant. After the supernatant was dislodged, proteinase K was added to precipitate to remove protein for DNA educing from DPC. DNA obtained above content (A) and DNA content in the supernatant (B) were detected by fluorometric method, and then the rate of crosslinked DNA / tota1 DNA was calculated, the crosslinks degree between DNA and protein was obtained. The formula was as followed:

$$\eta = A/(A+B)\times 100\%.$$

2.4. Make a Standard curve of DNA concentration

DNA was detected by Hoechst 33258. Calf thymus DNA standards were prepared at concentrations of 0, 100, 300, 500, 750, 1000, 1500, 3000, 5000 ng • mL-1. 1 mL of standard DNA was mixed with 1 mL of freshly prepared 400 ng · mL-1 Hoecst dye reagent of final concentration of 200 ng • mL-1. Precaution was taken to avoid expodure to light at this point, and the samples were placed in the dark for 30 min. Fluorescence of each sample was assessed by 970 CRT spectrophoto-fluorometer with exitation at 365 nm and emission at 450 nm. Regression equation of standard curve (Fig.1) as followed: $y=0.6410x+4.9392$, $r^2=0.9990$. Fluorescence of dyed samples were detected with 970 CRT spectrophotofluorometer, followed by quantitation of crosslinked DNA and free DNA on the basis of standard curve. At last, DPC coefficient (ŋ) was calculated.

2.5. Statistical analysis

The statistical significance of the difference between control and treated groups was evaluated by SPSS 13.0. A value of $P<0.05$ was considered statistically significant.

3. Results

3.1. Standard curve of DNA concentration

Fig.1 Standard curve of DNA concentrationm, Fluorescence of different concentration Calf thymus DNA Standards were detected is linear

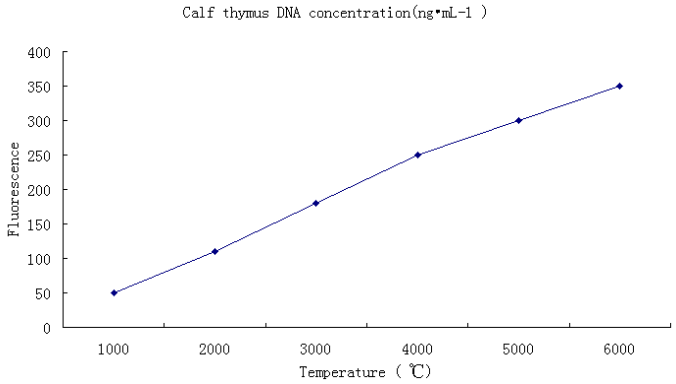

Fig.1 Standard curve of DNA concentration

3.2. Cr(VI) induced SPC-A1 cells could produce DNA-protein cross-linking

As shown in Table 1, All the different concentrations of Cr(VI) could cause the increase of DPC coefficient in SPC-A1 cells. Compared with the control group, but this effect was not significant ($P>0.05$) at low concentrations (2.5μmol/L); while in high concentration (10μmol/L, 40μmol/L, 160μmol/L) Cr(VI) induced SPC-A1 cells could produce DNA-protein cross-linking effect significantly ($P<0.05$).

Table 1. Cr(VI) induced SPC-A1 cells could produce DPC($\bar{x}\pm s$)

dose (μmol/L)	Combine DNA	Free DNA	DPC coefficient (%)
0	17.81±1.25	70.13±3.24	20.86±0.54
2.5	19..36±3.80	74.02±2.61	20.97±0.77
10	26.56±1.49	71.34±3.66	27.76±2.81
40	34.92±3.46	68.34±7.55	36.91±4.33
160	61.24±2.02	57.83±2.49	59.08±1.76

Compared with the control group, $P<0.05$.

4. Discussion

Toxic heavy metal contamination in the environment is a result of natural, industrial, and agricultural processes, and microorganisms inhabiting these contaminated environments utilize various mechanisms to reduce or eliminate metal toxicity. Chromium, mercury, cadmium, lead, and arsenic are among the most common toxic metal contaminants. Chromium is a widespread pollutant released mainly during industrial processing. There are a certain quantitative DPC in normal cells, which is normai connection or metabolism consequence between DNA and neucleo protein. But excess DPC is a pathological phenomenon, and also an important genetic damage of DNA [9]. DNA-protein crosslinks, an important genetic damage of environmental physical and chemical factors on bio-macromolecule, can act as a biomarker of genetic toxicants. Studies had found that DPC occurred

following exposure to a variety of environmental pollutants and chemical carcinogens, including formaldehyde, aldylagents, as compounds, some heavy metals such as chromium [10].

Hexavalent chromium [Cr(VI)] compounds have been considered as potent human carcinogens and have been shown to cause DNA damage including DNA-protein crosslinking in various cells and tissues[11].This study show that that lower concentration of Hexavalent chromium [Cr(VI)] compounds could not cause DNA-protein crosslinks, but higher concentration of Hexavalent chromium [Cr(VI)] compounds could DNA-protein crosslinks significantly. We conclude that our data indicate a possible mechanism of Hexavalent chromium carcinogenicity in humans and that DPC can be used as a method for biological monitoring of exposure to Hexavalent chromium. But in order to investigate whether DPC act as the biomarker of Hexavalent chromium genotoxic, further studies need to be done.

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