Chromium Incorporated in RNA and DNA

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The objective of this study was to examine the effect of Cr(III) (chromium chloride) and Cr(VI) (potassium dichromate) on RNA and DNA-chromium adducts formation in isolated nucleic acids and isolated pig lymphocytes. The incubation of cells with potassium dichromate and chromium chloride at concentrations of 10 and 100 μM results in binding of a 1.2–1.9 fold greater number of chromium atoms to nuclear DNA than to total cellular RNA. The incubation of total cellular RNA and nuclear DNA isolated from lymphocytes with $CrCl_3$ and $K_2Cr_2O_7$ yielded a binding of 1.1–1.6 fold more of Cr atoms to RNA than to DNA. The number of chromium atoms bound to nucleic acids is higher after incubation with $K_2Cr_2O_7$ than with $CrCl_3$ in both experimental systems.

Introduction

In aqueous solution chromium exists essentially in its trivalent (CrIII) and hexavalent (CrVI) forms, both cause chromium pollution. CrIII and CrVI have fundamentally different biochemical effects. Trivalent chromium compounds are non-toxic and some of them are essential in mammals for the maintenance of glucose, lipid and protein metabolism. Hexavalent chromium Cr(VI) is particularly dangerous because this form of chromium is easily and quickly transported into cells. When hexavalent chromium ions such as dichromate anion come in contact with organic substances or reducing agents it is reduced to the trivalent state, CrIII.

Chromium ions may induce mutations (Cheng *et al.*, 2000) or DNA damages (Blasiak *et al.*, 1999; Blasiak and Kowalik, 2000), or arrest of RNA (Maier *et al.*, 2000) and protein synthesis (Wang *et al.*, 1997).

The objective of the current study was to examine the effect of chromium ions on RNA-chromium cross-link formation in isolated RNA and isolated pig lymphocytes. Special care was taken on the comparison of chromium RNA and DNA damaging levels. The number of chromium atoms incorporated to nuclear DNA and total RNA were examined by atomic absorption spectrometry method.

Materials and Methods

Chemicals

Potassium dichromate, potassium chloride, sodium chloride, tris[hydroxymetyl]-aminomethane (Tris), guanidinium thiocyanate, phenol, sarcosyl, RPMI-1640 medium and chromium atomic absorption standard solution were obtained from Sigma Chemical Co (St. Louis, Mo, USA). All other chemicals were of highest purity available.

Cells

Lymphocytes were isolated from peripheral pig blood according to the modified method (Böyum, 1964). Cells were counted in a Bürker chamber. The viability of the cells was measured by trypan blue exclusion assay.

Preparation of RNA and DNA

Total RNA was isolated by a guanidinium-acid-phenol extraction (Chomczyñski and Sacchi, 1987). Following quantitation RNA samples were stored in suitable aliquots at –70 °C. Nuclear DNA was isolated by the phenol-detergent method of Kunkel *et al.* (1977) following quantitation DNA samples were stored in suitable aliquots at –20 °C.

Incubation conditions with potassium dichromate (K_2CrO_7) and chromium chloride ($CrCl_3$)

1.5 ml lymphocyte samples (1×10^7 cells/ml) were incubated with potassium dichromate or chromium chloride for 1 h at 37 °C. Cells were incubated with 10 μM and 100 μM chromium compounds in RPMI-1640 medium. Control cells were cultured without K_2CrO_7 and $CrCl_3$ in the same conditions. After incubation the cells were washed with phosphate buffered saline (0.01 M phosphate buffer, 0.0027 M potassium chloride, 0.137 M sodium chloride, pH 7.4).

200 μl DNA and RNA samples (0,1 mmol of nucleotides/ml) were incubated separately with potassium dichromate or chromium chloride in con-

centrations appointed as r_i coefficients (r_i-μmol of chromium compound added/μmol of nucleotides) equal 0.01 and 0.1 at 37^0C during one h in several replicates. After incubation nucleic acids were precipitated by adding of absolute ethanol and centrifuged at 10000 × *g* at 4 °C. Pellets were washed with 70% ethanol and dried. DNA and RNA were dissolved in sterile distilled water. The excess Cr compounds were removed by gel filtration on Sephadex G-25.

Statistical analysis was performed by t-Student method ($p < 0.05$).

Quantitation of chromium atoms bound to RNA and DNA

The number of atoms incorporated into RNA and DNA molecules were estimated by atomic absorption spectrometry. Nucleic acid samples were boiled in 14 M HNO_3 in a water bath during 30 min. Following mineralization the samples were diluted to a final HNO_3 concentration of 1% and the absorption was measured using a Varian Spectra A 300/400 spectrophotometer. Simultaneously series of chromium atomic absorption standard solutions were measured. The standard curve was prepared from a stock solution of chromium in 5% HCl. 1% HCl served as the control.

Results

Figure 1 presents a number of chromium atoms incorporated into DNA and RNA of pig lymphocytes after incubation of cells with 10 mM and 100 mM potassium dichromate and chromium chloride. Selected doses had no lethal activity on the cells investigated (data not shown). The number of Cr atoms bound to DNA molecules were significantly greater than bound to RNA and was 5.12 ± 0.30/1000 and 1.80 ± 0.11/1000 nucleotides for 10 μM potassium dichromate or chromium chloride and 7.74 ± 0.44/1000 nucleotides and 3.70 ± 0.25 for 100 μM respectively. Under the same conditions to RNA 3.86 ± 0.45 Cr atoms/1000 nucleotides and 1.04 ± 0.07 Cr atoms/1000 nucleotides were bound for 10 μM potassium dichromate or chromium chloride, 6.43 ± 0.59 Cr atoms /1000 nucleotides and 1.97 ± 0.37 Cr atoms/1000 nucleotides for 100 μM chromium compounds, respectively. Incubation of cells with potassium dichromate and chromium chloride at concentrations of 10 and 100 μM resulted in binding of 1.2–1.9 fold greater number of chromium atoms to nuclear DNA than to total cellular RNA.

Results of binding of chromium atoms after incubation of isolated nuclear DNA and total cellular RNA with Cr(III) (chromium chloride) and Cr(VI) (potassium dichromate) are shown on Figure 2. Both nucleic acids were incubated separately with $CrCl_3$ or $K_2Cr_2O_7$ in concentration coefficients r_i of 0.01 and 0.1. Numbers of Cr atoms bound to RNA were 50.00 ± 7.09/1000 nucleotides and 63.28 ± 6.22/1000 nucleotides for r_i = 0.01 chromium chloride and potassium dichromate and 69.61 ± 4.12/1000 nucleotides or 89.63 ±7.19/1000

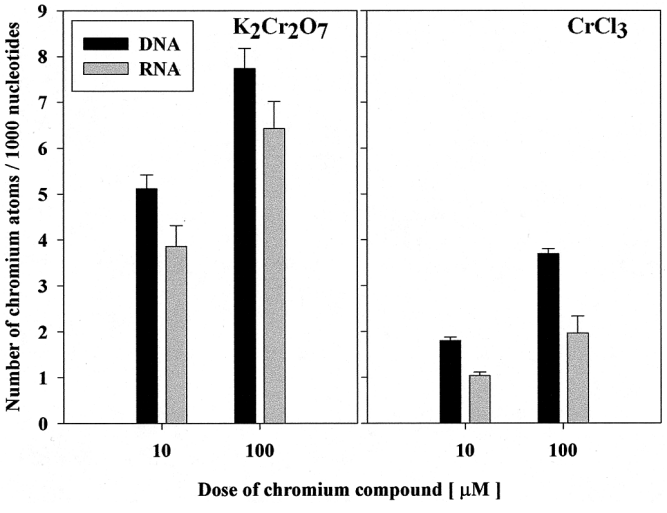

Fig. 1. The number of chromium atoms bound to 1000 nucleotides of nuclear DNA and total RNA after incubation of lymphocytes with potassium dichromate and chromium chloride as a function of compound concentration. Bars represent the mean ± SD.

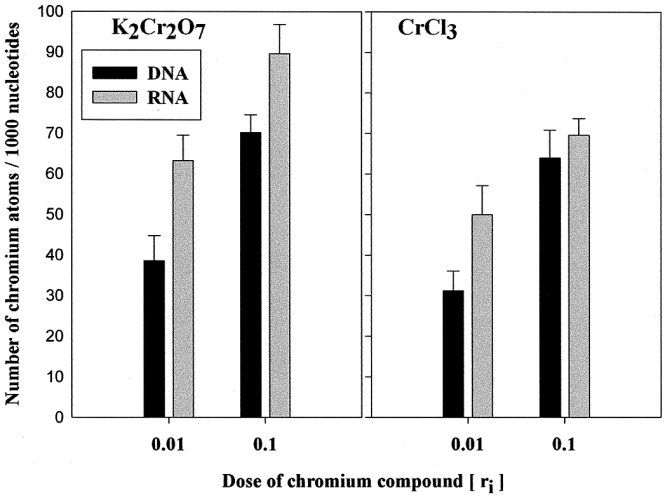

Fig. 2. The number of chromium atoms bound to 1000 nucleotides of nuclear DNA and total RNA after incubation of isolated nucleic acids with potassium dichromate and chromium chloride as a function of compound concentration appointed as r_i coefficients (r_i -µmol of chromium compound added/µmol of nucleotides). Bars represent the mean ± SD.

nucleotides for r_i = 0.1, respectively. In case of DNA estimated chromium atom numbers incorporated during incubation with chromium chloride were 31.25 ± 4.81/1000 nucleotides and 64.02 ± 6.85/1000 nucleotides for 0.01 and 0.1 $_i$ coefficients respectively. Incubation of isolated DNA with potassium dichromate resulted in incorporation of 38.52 ± 6.19 Cr atoms/1000 nucleotides for r_i = 0.01 and 70.21 for r_i = 0.1. No statistically essential differences were found between numbers of Cr atoms bound to DNA and RNA for r_i = 0.1. After incubation of isolated nucleic acids with a lower dose of chromium chloride and potassium dichromate we observed a greater number of chromium atoms bound to total RNA than to nuclear DNA. The incubation of total cellular RNA and nuclear DNA isolated from lymphocytes with $CrCl_3$ and $K_2Cr_2O_7$ with concentration coefficients r_i of 0.01 and 0.1 caused binding of 1.1–1.6 fold more of Cr atoms to RNA than to DNA.

The number of chromium atoms bound to nucleic acids was higher after incubation with $K_2Cr_2O_7$ than with $CrCl_3$ in both experimental systems.

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