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Short communication

Accumulation of chromium in Chinese hamster V79-cells and nuclei

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Abstract. The bioavailability of carcinogenic compounds plays an important role in the process of oncogenesis. Chromium in its hexavalent oxidation state is classified as carcinogen to humans. Therefore we studied the uptake of chromate(VI) into Chinese hamster V79 cells and nuclei isolated after the incubation of the whole cells with chromate. The chromium content of cells and nuclei was determined by atomic absorption spectroscopy. Chromate is taken up in a concentration dependent manner and accumulates to about 30 fold over the extracellular concentration of 0.1 mmol/l. Incubating whole cells with the same concentration results in an intranuclear concentration of up to 6 mmol/l after 3 h. The kinetic parameters (K_M = 0.34 mmol/l, V_{max} = 0.12 fmol per cell and minute) are in the same order of magnitude as previously published data. The consequences of the high intracellular and intranuclear concentrations are discussed in terms of the genotoxic effects.

Key words: Chromium(VI) – Uptake – Accumulation – V79 cells and nuclei

Introduction

Hexavalent chromium is classified as carcinogenic to humans (IARC 1987) but its exact mechanism has not been elucidated up to now. Soluble compounds of hexavalent chromium (Cr(VI)) are readily taken up by the anion exchange protein band-3 into erythrocytes (Ormos and Manyai 1977) and into nucleated mammalian cells (Debetto et al. 1988). In contrast, compounds of trivalent chromium compounds penetrate red cell membranes only to a very limited extent, since their uptake rate is about three orders of magnitude slower (Kortenkamp et al. 1987). The anion exchange is a passive transport process independent of metabolic energy and controlling both influx and efflux of inorganic anions (Schnell et al. 1977). With the chromate anion, however, no efflux has been observed (Kitagawa et al. 1982). This intracellular trapping of chromium is due to the rapid reduction of Cr(VI) by cellular constituents (Connett and Wetterhahn 1983).

Though the uptake and bioavailability of chromium plays an important role in chromium toxicity, an accumulation of the metal in nucleated cells and in nuclei *in vitro* has not yet been investigated in detail. Langård (1979) demonstrated that *in vivo* after intravenous administration considerable amounts of chromium can be found in the nuclei of rat liver. Rat thymocytes *in vitro* readily take up Cr(VI) but an intracellular accumulation has not been observed (Debetto et al. 1988). In the present study we report that in Chinese hamster V79 cells chromium accumulates in a concentration dependent manner which results in very high concentrations of chromium in the nuclei.

Materials and methods

Chemicals. Water was twice distilled over quartz. All inorganic salts were of reagent grade (p.a.) from Merck (Darmstadt, FRG). Concentrated nitric acid and 33% H_2O_2 were of suprapure quality obtained from Merck (Darmstadt, FRG). The chromate stock solution (10 mM Na_2CrO_4) was prepared in water and diluted with buffered saline (PBS: 136.9 mM NaCl, 2.7 mM KCl, 8.1 mM $Na_2HPO_4 \cdot 2H_2O$; 1.5 mM KH_2PO_4, pH 7.2) to the appropriate concentrations. Isoton II was supplied by Coulter Electronics (Krefeld, FRG).

Cell culture. Chinese Hamster V79 cells were obtained from Dr Detlef Gabel of the same department, University of Bremen and grown as described previously (Hartwig and Beyersmann 1987).

Isolation of nuclei. Nuclei were isolated using a modification of the method described by Richter-Landsberg et al. (1985). Briefly, the medium was aspirated and the cells were rinsed with PBS. Five minutes after cell lysis with 1 ml non-ionic detergent (0.5% Nonidet-P40) in nuclei extraction buffer (10 mM TRIS-HCl, 3 mM $MgCl_2$, 10 mM NaCl, pH 7.5) 1 ml of the same buffer was added, and the nuclei carefully but thoroughly mixed and counted.

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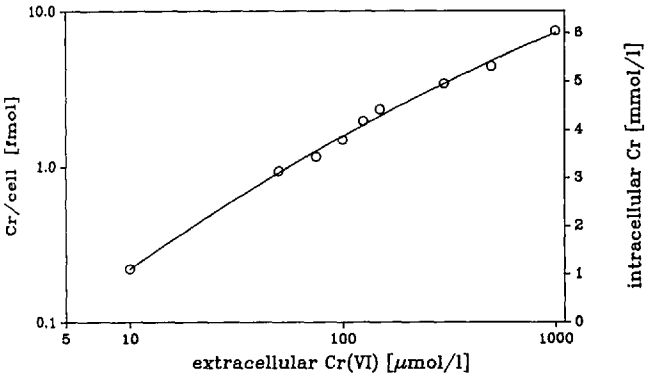

Fig. 1. Concentration dependent uptake of chromate(VI) by V79 cells. Cells were incubated with the indicated concentrations of Na_2CrO_4 and after 1 h the medium was aspirated and the cells rinsed twice with ice-cold PBS. After trypsinizing and counting the chromium content per cell was determined by AAS

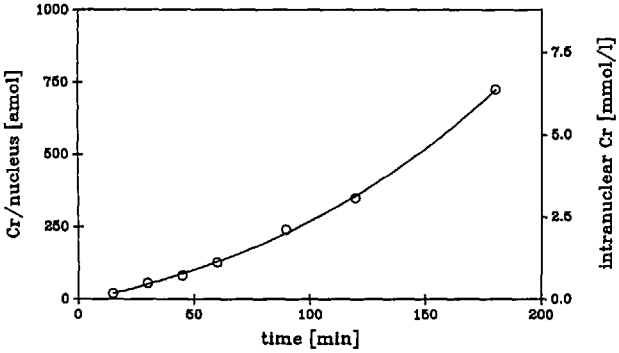

Fig. 3. Time dependent accumulation of chromium in V79 nuclei. Cells were incubated with 100 μmol/l Na_2CrO_4. After the times indicated the nuclei were isolated and the chromium content per nucleus determined by AAS

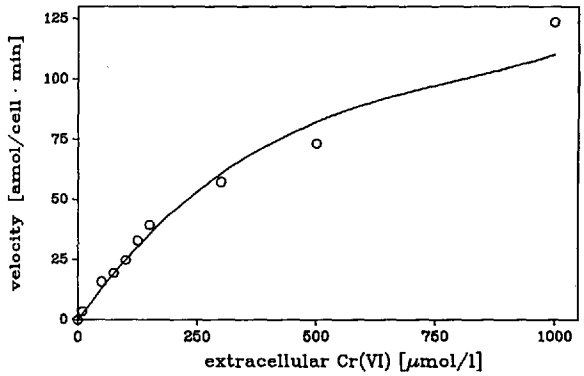

Fig. 2. Michaelis-Menten plot of the transport kinetics of chromate(VI) into V79 cells. Velocities were calculated from the data shown in Fig. 1.

Determination of the volume of cells and nuclei. Trypsinized V79 cells or isolated nuclei were washed with PBS and resuspended in isotonic electrolyte solution (Isoton II). Cell numbers and cell sizes were determined in a Coulter Counter, equipped with a partical size analyser. Volumes were related to the highest median and calculated as described previously (Buttner et al. 1988).

Incubation with chromium compounds and metal determination. V79 cells were inoculated in duplicate (or triplicate for controls) into 60 mm dishes, grown for 24 h, supplied with fresh medium containing different concentrations of Cr(VI) and incubated for the times indicated below. The uptake of chromium was stopped by washing the cells twice with 5 ml ice-cold PBS. Washed cells or isolated nuclei were centrifuged at 12800 *g* for 1 min, and the pellets were mineralized and analyzed for chromium by atomic absorption spectrometry (AAS) as described previously (Kortenkamp et al. 1987).

Results

The uptake of chromate(VI) and the accumulation of chromium in Chinese hamster cells was studied by graphite furnace atomic absorption spectroscopy.

In order to calculate the chromium concentration within cells and nuclei their volumes were determined in a Coulter Counter to 1.55 10^{-12}l and 0.11 10^{-12}l, respectively. An important factor in the determination of the cell volumes is the shape of the cells and/or nuclei passing through the orifice. As the microscopic evaluation revealed a spherical shape for the trypsinized cells and of the isolated nuclei, the calibration of the method with spherical Latex particles appears adequate. The analysis of the cells in the Coulter Counter also showed that under our experimental conditions the cells were physically stable.

The uptake of chromium by V79 cells increased linearly with the chromate concentration applied, and even at 1 mmol/l Cr(VI) no plateau was reached in the intracellular chromium concentration, which was 5-fold over that applied extracellularly (Fig. 1). Since within the time scale (1 h) the uptake rate was still independent of the time (not shown), we could subject our data to a Michaelis-Menten type kinetic analysis (Fig. 2). The kinetic parameters were estimated by the direct linear plot of Eisenthal and Cornish-Bowden (1974) and the corresponding computer program of Brady and Ishizaki (1989). K_M was found to be 0.34 mmol/l and V_{max} was 0.12 fmol per cell and minute.

Finally, we studied the uptake of chromium into nuclei of the V79 cells, since this is an important factor in the evaluation of the genotoxicity of the carcinogen chromate. V79 cells were incubated with 0.1 mmol/l Na_2CrO_4, cell nuclei were isolated 10–180 min after the addition of chromate, and the intranuclear chromium was analyzed (Fig. 3). We observed increasing nuclear levels of chromium, which reached 6 mmol/l (right ordinate in Fig. 3), i.e. a 60-fold accumulation over the extracellular chromate concentration within 3 h.

Discussion

Our results show that Chinese hamster V79 cells take up chromate(VI) with similar kinetic parameters to other cell types studied previously. Table 1 summarizes the V_{max} and K_M data reported. The maximal uptake rates are of the same order of magnitude in human erythrocytes and V79 cells. The Michaelis constants reported (Table 1)

Table 1. Kinetic parameters of chromate uptake by mammalian cells reported in various studies

Cell type	V_{max}	K_M
Human erythrocytes		
Wiegand et al. (1985)	$5.2 \cdot 10^{-16} \frac{mol}{cell \cdot min}$ [a]	
Buttner and Beyersmann (1985)	$2.2 \cdot 10^{-18} \frac{mol}{cell \cdot min}$ [bc]	5 µmol/l[bc]
	$1.4 \cdot 10^{-16} \frac{mol}{cell \cdot min}$ [bd]	892 µmol/l[bd]
Rat erythrocytes		
Wiegand et al. (1985)	$4.2 \cdot 10^{-16} \frac{mol}{cell \cdot min}$ [a]	
Rat thymocytes		
Debetto et al. (1986)	$8.5 \frac{nmol}{mg\ protein \cdot 15\ min}$	63 µmol/l
Debetto et al. (1988)	$6.8 \frac{nmol}{mg\ protein \cdot 20\ min}$	540 µmol/l
V79 cells		
This study	$1.2 \cdot 10^{-16} \frac{mol}{cell \cdot min}$	340 µmol/l

[a] Value calculated from Wiegand's V_{max} given in $\frac{ions}{cell \cdot min}$

[b] The value published was erroneous
The correct value shown here was obtained by recalculation of the original data

[c] In the concentration range 1 – 10 µmol/l extracellular Cr(VI)

[d] In the concentration range 25 – 1000 µmol/l extracellular Cr(VI)

differ by 1 order of magnitude even within the same cell type and laboratory. Since K_M is different for low and high extracellular chromate concentrations (Buttner and Beyersmann 1985), we compare the data obtained from the upper chromate range only and find a good agreement between the K_M values for human red blood cells, rat thymocytes and Chinese hamster V79 cells (Table 1).

Our results also show that Chinese hamster V79 cells accumulate chromium both in the whole cells and in the nuclei isolated after incubation of the cells with chromate. This accumulation is explained by the uptake-reduction model (Connett and Wetterhahn 1983): the chromate anion penetrates cell membranes via the general anion carrier, and it is reduced intracellularly by cytoplasmic constituents. These are trapped within the cells, since they are not able to pass the plasma membrane.

The high concentration of chromium accumulated in cell nuclei after uptake of chromate certainly contributes to the genotoxic effects observed, since trivalent chromium efficiently binds to isolated cell nuclei (Köster and Beyersmann 1985) and interacts specifically with nucleotides and nucleic acids (Denniston and Uyeki 1987; Wolf et al. 1989). The high intracellular chromium concentration seems to be essential to overcome inactivating protecting reactions that reduce and bind chromium before it is able to react with DNA immediately or via reactive metabolites.

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