衛生化学
EISEI KAGAKU
32 (2) 123-127 (1986)

Forms of Chromium in Rat Blood Treated *in Vivo* and *in Vitro* with $\text{Na}_2^{51}\text{CrO}_4$ and $^{51}\text{CrCl}_3$

KATSUHIKO NAKAMURO and YASUYOSHI SAYATO

Faculty of Pharmaceutical Sciences, Setsunan University, 45-1,
Nagaotoge-cho, Hirakata, Osaka 573-01, Japan

(Received, October 29, 1985)

The forms of chromium and the interaction of radiolabelled chromium chloride and sodium chromate with the constituents of red blood cells and plasma *in vivo* and *in vitro* were investigated by Sephadex gel chromatographic techniques. The major chemical form in plasma treated with trivalent chromium was chromium-binding protein, and that in plasma treated with hexavalent chromium was a low molecular chromium complex possessing high ability to permeate through the red cell membrane. The form of chromium in stroma-free hemolysate after hexavalent chromium treatment was considered to be a chromium complex which bound easily to hemoglobin. After trivalent chromium treatment, a chromium complex binding easily to higher molecular proteins than hemoglobin was found in stroma-free hemolysate.

Keywords—existing chemical form; hexavalent chromium; trivalent chromium; rat blood; plasma; stroma-free hemolysate; red cell permeability; gel filtration

Introduction

Chromium is an environmental pollutant, and chromium compounds are extensively used in industry and commerce. The influence of chromium compounds on biological systems is therefore important from the standpoint of environmental toxicology.

We have already reported comparative studies on the metabolic fate of chromium in rats after administration of $\text{Na}_2^{51}\text{CrO}_4$ and $^{51}\text{CrCl}_3$.¹⁾ It was concluded that the metabolic fate of chromium in rats depends on the facts that trivalent chromium possesses a strong binding affinity for transferrin in the plasma, while hexavalent chromium permeates through red cell membranes and binds to hemoglobin.

Several researchers²⁻⁸⁾ have investigated the binding behavior of chromium with the blood constituents. These reports⁶⁻⁸⁾ have stated that hexavalent chromium penetrates into red cells

rapidly without appreciable reaction with constituents of the plasma, and that the hexavalent form is reduced to the trivalent form by reduced glutathione⁸⁾ inside the cells and subsequently bound to hemoglobin. On the other hand, the trivalent form was firmly bound to the plasma protein transferrin³⁾ without migrating into red cells.

However, little information is available on the comparative interaction of trivalent and hexavalent chromium with the blood constituents. The present paper describes the forms and the interactions of trivalent and hexavalent chromium in rat red blood cells and plasma.

Materials and Methods

Animals and Materials—Male Wistar strain rats, weighing about 200 g, were used in these experiments. Each rat was housed under the same conditions as described in a previous report.¹⁾

Radioactive $^{51}\text{CrCl}_3$ and $\text{Na}_2^{51}\text{CrO}_4$ were purchased from the Radiochemical Centre (Amersham, U.K.) and diluted with non-radioactive solutions of CrCl_3 and Na_2CrO_4 , respectively, in physiological saline or distilled water. Human hemoglobin ($\times 2$ crystallized) was purchased from ICN Pharmaceuticals Inc. and human transferrin from Miles Laboratories, Inc. (U.S.A.). Other chemicals were obtained from commercial sources.

Incubation of Rat Blood and Blood Components with $^{51}\text{CrCl}_3$ and $\text{Na}_2^{51}\text{CrO}_4$ *in Vitro*—

Blood samples were obtained by cardiac puncture with a heparinized syringe, and the plasma was immediately separated from blood cells by centrifugation. The red cells were washed three times with physiological saline and centrifuged at 1500 *g* for 5 min. After the final wash, the red cells were lysed by adding water. The stroma-free hemolysate was obtained by centrifugation at 20000 *g* for 10 min to remove the red cell membranes. The fractionation was performed at 0–5°C.

Whole blood, suspension of red cells in physiological saline instead of plasma, stroma-free hemolysate, human hemoglobin and human transferrin were used for this experiment. These samples were incubated with an aliquot of 2.5×10^7 cpm/0.02 mg Cr/ml $^{51}\text{CrCl}_3$ or $\text{Na}_2^{51}\text{CrO}_4$ solution for 1 or 6 h at 37°C. After incubation, these treated samples were used for the experiments on distribution in blood components and Sephadex G-200 gel chromatography.

Sephadex Gel Filtration—The plasma and stroma-free hemolysate were prepared from rats 15 min, 2 h and 6 h after i.v. injection of 2.5×10^7 cpm/0.02 mg Cr/kg of $^{51}\text{CrCl}_3$ or $\text{Na}_2^{51}\text{CrO}_4$ solution. These samples and *in vitro*-treated samples described above were applied to a column (1.8 $\times$ 50 cm) of Sephadex G-200 and the column was eluted with 0.9% NaCl solution at a flow rate of approximately 10 ml/h. The radioactivity and absorbance at 254 nm of each fraction (2 ml) were determined. Radioactivities were counted in a well-type scintillation counter (Aloka model P 100). Radioactivity of ^{51}Cr recovered was about 90% in all cases.

Hexavalent chromium in the fractions was deter-

mined by a modification of the tri-*n*-octylamine-methylisobutylketone (TOA-MIBK) extract method.⁹⁾ The radioactivity of MIBK extracts were measured with a well-type scintillation counter (Aloka model P 100).

Results and Discussion

1. Distribution of Chromium in Blood Components *in Vitro* and *in Vivo*

Distribution of chromium in blood components were investigated *in vivo* and *in vitro* in order to clarify the interactions of $^{51}\text{CrCl}_3$ and $\text{Na}_2^{51}\text{CrO}_4$ with rat blood. Figure 1 shows the distribution of ^{51}Cr in rat blood components 6 h after i.v. injection and *in vitro* treatment with both forms of chromium at 37°C.

Most of the $^{51}\text{Cr}^{3+}$ after *in vivo* treatment was distributed in the plasma because of its strong affinity for high molecular proteins,^{1,3)} but a considerable amount of $^{51}\text{Cr}^{3+}$ after *in vitro* treatment was distributed in stroma-free hemolysate. The ability of $^{51}\text{Cr}^{3+}$ to permeate into red blood cells suspended in physiological saline instead of plasma was higher than that in the case of whole blood *in vitro*. The permeation of $^{51}\text{Cr}^{6+}$ *in vitro* was similar to that *in vivo*. These results *in vitro* suggest that inorganic trivalent and hexavalent chromiums can permeate through the naked red blood cell membrane in the absence of the plasma.

2. Form of Chromium in the Plasma

Figure 2 shows Sephadex G-200 gel filtration profiles of the plasma prepared from rats 15 min, 2 h, 6 h and 5 d after i.v. injection of $^{51}\text{CrCl}_3$ or $\text{Na}_2^{51}\text{CrO}_4$. $^{51}\text{Cr}^{3+}$ in transferrin (reported by Hopkins and Schwarz³⁾) contained in albumin fractions reached the maximum level 2 h after i.v. injection of $^{51}\text{CrCl}_3$, then decreased. However, 15 min after injection of $^{51}\text{CrCl}_3$, $^{51}\text{Cr}^{3+}$ appeared in proteins with higher molecular weight than albumin. The $^{51}\text{Cr}^{3+}$ in low molecular fractions decreased rapidly. In the $\text{Na}_2^{51}\text{CrO}_4$ group, $^{51}\text{Cr}^{6+}$ in

Fig. 1. Distribution of ^{51}Cr in Rat Blood Components 6 h after i.v. Injection and after *in vitro* Incubation for 6 h with $^{51}\text{CrCl}_3$ or $\text{Na}_2^{51}\text{CrO}_4$

□ : plasma, ▨ : stroma-free hemolysate, ▩ : stroma.

1) Suspension of red blood cells in physiological saline instead of plasma.

2) This column shows physiological saline fractions.

Fig. 2. Sephadex G-200 Chromatograms of Rat Plasma after i.v. Injection of $^{51}\text{CrCl}_3$ or $\text{Na}_2^{51}\text{CrO}_4$

— : 15 min, ---- : 2 h, : 6 h, - · - · : 5 d.

●—● : Typical pattern of 254 nm OD.

albumin fractions and low molecular fractions decreased rapidly.

In order to identify the forms of chromium

TABLE I. Detection of Hexavalent Chromium in Low Molecular Fractions by the TOA-MIBK Extract Method

Compounds	Treatment Blood components	Amount of ^{51}Cr (%)	
		MIBK layer ^{a)}	Water layer ^{b)}
$^{51}\text{CrCl}_3$	— ^{c)}	0.7	99.3
	Plasma	0.6	99.4
	Stroma-free hemolysate	3.8	96.2
$\text{Na}_2^{51}\text{CrO}_4$	— ^{c)}	93.6	6.4
	Plasma	1.1	98.9
	Stroma-free hemolysate	4.8	95.2

a) The MIBK layer contains hexavalent chromium.

b) The water layer contains ^{51}Cr other than hexavalent chromium.

c) The water solution of $^{51}\text{CrCl}_3$ or $\text{Na}_2^{51}\text{CrO}_4$ was used without addition of blood components.

Each value is the mean of 2 or 3 experiments.

Rat blood 6 h after i.v. injection of $^{51}\text{CrCl}_3$ or $\text{Na}_2^{51}\text{CrO}_4$ was used.

in low molecular fractions separated by Sephadex G-200 gel filtration of the plasma and stroma-free hemolysate, $^{51}\text{Cr}^{6+}$ in these fractions was determined by the TOA-MIBK extract method. As shown in Table I, no $^{51}\text{Cr}^{6+}$ was detected in low molecular fractions of the plasma or stroma-free hemolysate. It is assumed from these results that hexavalent chro-

TABLE II. Detection of Chromium in Sephadex G-200 Gel Filtration Fractions of Blood Components Incubated with $^{51}\text{CrCl}_3$ or $\text{Na}_2^{51}\text{CrO}_4$

Sample for gel filtration	Incubation		Fractions separated by gel filtration	
	Component	Compound	Hemoglobin	Low molecular fraction
Stroma-free hemolysate	Whole blood	$^{51}\text{CrCl}_3$	— ^{b)}	+ ^{c)}
	Whole blood	$\text{Na}_2^{51}\text{CrO}_4$	+	+
	Red cells	$^{51}\text{CrCl}_3$	—	+
	Red cells	$\text{Na}_2^{51}\text{CrO}_4$	+	+
	S.F.H. ^{a)}	$^{51}\text{CrCl}_3$	—	+
	S.F.H.	$\text{Na}_2^{51}\text{CrO}_4$	—	+
Human hemoglobin	Human hemoglobin	$^{51}\text{CrCl}_3$	+	
	Human hemoglobin	$\text{Na}_2^{51}\text{CrO}_4$	—	

a) S.F.H. is stroma-free hemolysate.

b) — : The radioactivity of ^{51}Cr was not detected in fractions separated by Sephadex G-200 gel filtration.

c) + : The radioactivity of ^{51}Cr was detected in fractions separated by Sephadex G-200 gel filtration.

mium was converted to Cr^{3+} ion or chromium complex.

A further investigation was undertaken to study whether $^{51}\text{Cr}^{3+}$ and $^{51}\text{Cr}^{6+}$ are bound *in vitro* to plasma protein and human transferrin. Whole blood and human transferrin were each incubated with $^{51}\text{CrCl}_3$ or $\text{Na}_2^{51}\text{CrO}_4$ for 1 h at 37°C . The plasma separated from whole blood and the human transferrin were each subjected to Sephadex G-200 gel chromatography. The ^{51}Cr in the plasma after incubation with both forms of chromiums *in vitro* was bound to transferrin in albumin fractions, while human transferrin seemed to bind only $^{51}\text{Cr}^{3+}$, but not $^{51}\text{Cr}^{6+}$.

These observations indicate that when rat blood was treated with $\text{Na}_2^{51}\text{CrO}_4$, one of the chemical form of chromium in the plasma may be chromium binding transferrin in albumin fractions and another may be trivalent chromium complex. If the hexavalent chromium is converted directly into trivalent chromium in the plasma, the two forms of chromium should show the same behavior as $^{51}\text{Cr}^{3+}$ in the plasma. However, $\text{Na}_2^{51}\text{CrO}_4$ possessed high ability to permeate through the red cell membrane and to bind to hemoglobin.^{1,6,7)} Therefore, it is considered that the chemical form of

Fig. 3. Sephadex G-200 Chromatograms of Rat Stroma-Free Hemolysate after i.v. Injection of $^{51}\text{CrCl}_3$ or $\text{Na}_2^{51}\text{CrO}_4$

— : 15 min, --- : 2 h, : 6 h.

●—● : Typical pattern of 254 nm OD.

chromium in the plasma treated with $\text{Na}_2^{51}\text{CrO}_4$ should be a lower molecular chromium complex that is able to permeate through the red cell membrane.

3. Form of Chromium in Stroma-Free Hemolysate

Gabrieli and Heckert,⁶⁾ Carter *et al.*⁷⁾ and Aaseth *et al.*⁸⁾ have stated that hexavalent

chromium (CrO_4^{2-}) penetrates into red cells rapidly, the hexavalent form is reduced to the trivalent form by reduced glutathione in the cell *in vitro*,⁸⁾ and then trivalent chromium binds directly to hemoglobin.

The forms of chromium in stroma-free hemolysate after the penetration of $^{51}\text{CrCl}_3$ and $\text{Na}_2^{51}\text{CrO}_4$ through the red blood cell membrane were investigated. Figure 3 shows the Sephadex G-200 gel filtration profiles of the stroma-free hemolysate prepared from rats 15 min, 2 h and 6 h after i.v. injection of $^{51}\text{CrCl}_3$ or $\text{Na}_2^{51}\text{CrO}_4$.

As shown in Fig. 3, most ^{51}Cr was bound to hemoglobin in the $\text{Na}_2^{51}\text{CrO}_4$ group, but in the $^{51}\text{CrCl}_3$ group ^{51}Cr was found in higher molecular weight proteins than hemoglobin. The ^{51}Cr in both groups decreased rapidly in both high and low molecular fractions and no ^{51}Cr radioactivity was detected 2 h after injection.

Table II. shows the result of Sephadex G-200 gel filtration of blood components treated with $^{51}\text{CrCl}_3$ and $\text{Na}_2^{51}\text{CrO}_4$ *in vitro*. Whole blood, red blood cells suspended in physiological saline instead of plasma, separated stroma-free hemolysate and human hemoglobin were incubated with $^{51}\text{CrCl}_3$ or $\text{Na}_2^{51}\text{CrO}_4$ for 1 h at 37°C . Gel filtration indicated that hexavalent chromium was not bound to human

hemoglobin or hemoglobin in the separated stroma-free hemolysate, but was bound to hemoglobin in whole blood and separated red blood cells. On the other hand, trivalent chromium was bound to human hemoglobin, but not to hemoglobin in the whole blood, the separated red blood cells stroma-free hemolysate. When separated stroma-free hemolysate was treated with $^{51}\text{CrCl}_3$, a small amount of ^{51}Cr was bound to higher weight proteins than hemoglobin.

In contrast to the results of Gabrieli and Heckert⁶⁾ and Carter *et al.*⁷⁾, the form of chromium in stroma-free hemolysate of the rat blood treated with $\text{Na}_2^{51}\text{CrO}_4$ was considered to be a chromium complex able to bind strongly to hemoglobin, because $^{51}\text{Cr}^{6+}$ was not directly bound to human hemoglobin or hemoglobin in stroma-free hemolysate, but was bound to hemoglobin in the whole blood and separated red blood cells.

On the other hand, the form of chromium in stroma-free hemolysate of rat blood treated with $^{51}\text{CrCl}_3$ might be a chromium complex able to bind to higher molecular weight proteins than hemoglobin because $^{51}\text{Cr}^{3+}$ was bound to human hemoglobin, but was not bound to hemoglobin in whole blood treated with $^{51}\text{CrCl}_3$ *in vivo* and *in vitro*.

References

- 1) Y. Sayato, K. Nakamuro, S. Matsui and M. Ando, *J. Pharmacobio-Dyn.*, **3**, 17 (1980).
- 2) E.J. Underwood, "Trace Elements in Human and Animal Nutrition" 3rd ed., Academic Press, New York, 1971, pp.253.
- 3) L.L. Hopkins Jr. and K. Schwarz, *Biochim. Biophys. Acta*, **90**, 484 (1964).
- 4) S.J. Gray and K. Sterling, *J. Clin. Invest. Derm.*, **41**, 111 (1964).
- 5) H.K. Prins, *Vox Sang.*, **7**, 370 (1962).
- 6) E.R. Gabrieli, P. Heckert, *Proc. Soc. Exp. Biol. Med.*, **113**, 206 (1963).
- 7) E.B. Carter, D.F. Jackson and A.R. Kolber, *J. Appl. Radiation Isotopes*, **18**, 615 (1967).
- 8) J. Aaseth, J. Alexander and T. Norseth, *Acta Pharmacol. et Toxicol.*, **50**, 310 (1982).
- 9) T. Shimada, K. Tanaka, S. Fukui and S. Kanno, *Eisei Kagaku*, **19**, 35 (1973).