Valence States of Plasma Chromium in Rats after Intraperitoneal Administration of Sodium Chromate

Key words: Hexavalent and trivalent chromium—Reduction—Plasma—Reducing capacity

It is well known that soluble chromates, when added to blood in vitro or in vivo, hardly interact with the plasma components and easily penetrate the erythrocytes, and that reduction to the trivalent species follows. However, in vitro reduction of chromium(VI) in human and rat plasma has been observed. Some problems of the fate of chromium(VI) in blood, especially in vivo remain unsolved, because of the difficulty in determining this chromium species in biological samples. For studying these problems, suitable analytical methods for the determination of chromium(VI) in biological samples are required.

Recently, high-performance liquid chromatography (HPLC) with an anion-exchange column (FPLC system with Mono Q HR 5/5, Pharmacia, Sweden) was reported to be useful for the determination of water-soluble chromium(VI). Using this method, the reduction of chromium(VI) in plasma, erythrocyte lysate and soluble fractions of the livers of rats has been examined after addition of sodium chromate to the samples in vitro. In the present experiment, the plasma of rats injected intraperitoneally (ip) with Na₂CrO₄ was examined for the presence of chromium(VI), using this HPLC method. The fate of this chromium species in blood will be also discussed.

Male adult rats (360–430 g) of the Sprague-Dawley strain (Clea Japan, Tokyo, Japan), fed on a commercial pelleted diet (CE-2, Clea Japan) and sterilized tap water ad libitum, were injected with a saline solution [1 mg chromium(VI)/ml, 5 mg chromium(VI)/kg body weight] of Na₂CrO₄·4H₂O (analytical reagent grade, J. T. Baker, Phillipsburg, Nj, U.S.A.). Blood was taken from the hearts of Nembutal-anesthetized animals with heparinized syringes at 5, 10, 20, 40 and 60 min after injection. The blood was centrifuged at 3000 rpm for 10 min at 4°C and the plasma was separated. The plasma samples were immediately analyzed for chromium(VI) by the anion-exchange HPLC described above. The eluates were also examined for total chromium by atomic absorption spectrophotometry (AAS) after fractionation. These operations were performed as rapidly as possible. Aliquots of the whole blood and plasma samples were digested in nitric acid and hydrogen peroxide on a hot plate. The residues were dissolved in dilut nitric acid and analyzed for chromium by AAS.

Fig. 1 shows typical HPLC profiles of the plasma sample 10 min after chromium(VI) injection. A small new absorption peak (shadowed) was revealed by absorbance at 370 nm (Fig. 1B). The elution time of this peak was the same
as that of chromium(VI). In consistency with this peak, AAS showed a chromium peak at fraction 16, as seen in Fig. 1C. These observations indicate that the new absorption peak was due to chromium(VI). The other chromium signals including the highest (fraction 9) and minor peaks might be due to chromium(III) complexes and colloidal hydroxides of chromium(III). These HPLC profiles are substantially consistent with those obtained from rat plasma treated with chromium(VI) in vitro. The present findings clearly demonstrate that after ip
injection of Na₂CrO₄, this metal was released into the blood as chromate anions and that some of these anions were reduced to the trivalent species in the plasma.

Several separation peaks of components of the plasma were also revealed by absorption at 280 nm (Fig. 1A), but only albumin was identified.⁵)

As shown in Fig. 2, in the chromium(VI)-treated animals, the plasma concentrations of chromium(VI) increased rapidly at first, and reached the highest levels 10 min after injection. Thereafter, the chromium(VI) levels decreased and only the traces were observed 40 min after injection. The maximum chromium(VI) concentration in the plasma was 0.38 µg/ml. Total chromium in the plasma and whole blood showed the highest concentrations of 5.8 and 10.7 µg/ml at 40 min after injection, respectively. On the other hand, the ratios between chromium(VI) and total chromium in the plasma were highest in the first determination (5 min after injection) as shown in Table 1. However, even at this early time, chromium(VI) in the plasma was only 11% of the total chromium, indicating that most of the chromium in the plasma was the trivalent species.

In this experiment, chromium(VI) was identified in the plasma of rats after ip injection of Na₂CrO₄. However, higher levels of chromium(III) were simultaneously observed in the plasma even in the early period after injection. On the basis of the results of the chromium(VI) reduction study in plasma by Suzuki,⁶) these findings could be elucidated by immediate reduction of chromium(VI) in the plasma. The author has reported that the capacity of rat plasma for reducing
chromium(VI) is 1.5 μg/ml in vitro. Korallus et al.4) have also reported that chromium(VI) is reduced in human plasma in vitro and that its reducing capacity is about 2 μg/ml of chromium(VI). They have revealed that ascorbic acid is an important reducing factor in human plasma. In addition to ascorbic acid, glutathione2,7) and sulfhydryl-containing proteins8) might be involved in the reduction of chromium(VI) in the plasma. These reductants would be depleted in the reduction reaction of chromium(VI) in the plasma, when excess chromium(VI) were supplied.

From the maximum levels of plasma chromium, most of which was the trivalent species, the capacity of the plasma for reducing chromium(VI) in vivo was estimated to be approximately 5 μg/ml, clearly higher than those obtained in the in vitro experiments.4,5) This discrepancy might be due to the supplementation of the depleted reductants in the plasma which would operate in vivo, unlike the in vitro reduction. The supplementary reductants, probably supplied from the erythrocytes and organs, would enable the plasma to be more active in reduction. Furthermore, this discrepancy suggests that chromium(III) and/or its complexes produced in the erythrocytes,1,2,7) and organs9,10) and probably also in the abdominal cavity may be released into the plasma. Complexes of chromium(III) with glutathione11) and low-molecular-weight substances12) may be involved in the release of chromium(III).

In vitro study4) has suggested that ascorbic acid therapy could be effective in preventing acute chromium(VI) poisoning by exposure of the skin to soluble chromates, because of its ability to reduction of chromium(VI) to inactive chromium(III) in the plasma. The activated reduction of chromium(VI) in the plasma shown in the present in vivo experiment is interesting and further studies on the mechanism will be necessary.

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Table 1. Ratios of chromium(VI) in the plasma to total chromium in the plasma and whole blood of rats after ip injection of Na2CrO4

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<tr>
<th>After injection (min)</th>
<th>% Ratiosa of plasma chromium(VI) to total chromium in</th>
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<tr>
<td></td>
<td>Plasma</td>
<td>Whole blood</td>
</tr>
<tr>
<td>5</td>
<td>11.3</td>
<td>7.8</td>
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<tr>
<td>10</td>
<td>10.1</td>
<td>7.7</td>
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<td>20</td>
<td>2.2</td>
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<td>40</td>
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a Means of 5 animals.
REFERENCES


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