Binding of Chromium to Chromatin and DNA from Liver and Kidney of Rats Treated with Sodium Dichromate and Chromium(III) Chloride in Vivo

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ABSTRACT

The in vivo binding of chromium to whole chromatin, polyribosomes, DNA, and cytoplasmic RNA-protein fraction from liver and kidney was examined after treatment of rats with sodium dichromate and chromium(III) chloride. Significant amounts of chromium were bound to DNA and the nonhistone proteins of chromatin and to cytoplasmic RNA-protein fraction. The binding of chromium to the nuclear and cytoplasmic nucleic acid fractions varied considerably, depending on the tissue and the oxidation state of the chromium administered. The level of chromium bound to whole chromatin was greater in the liver than in the kidney after treatment with either chromium compound. Chromium entered the liver and kidney tissues at a slower rate after chromium(III) treatment than after chromium(VI) treatment. At early times after chromium(VI) treatment, more chromium was bound to the liver and kidney chromatin and DNA than after chromium(III) treatment. A much smaller proportion of the chromium bound to chromatin was associated with the DNA after treatment with chromium(III) than after treatment with chromium(VI). However, 40 hr after injection, there was no significant difference in the level of chromium on the DNA from both the liver and kidney of chromium(VI)- and chromium(III)-treated animals. No DNA damage was detected in either liver or kidney nuclei after chromium(III) treatment, using the technique of alkaline elution. A possible correlation between chromium binding to chromatin and DNA damage is discussed.

INTRODUCTION

The physiological effects of chromium compounds have been studied extensively because human occupational exposure to many of these compounds is widespread (21). Exposure of humans to chromium(VI) compounds has resulted in renal necrosis, hepatic damage, and respiratory cancer (7, 14). In animals, administration of chromium(VI) compounds by injection and implantation resulted in tumors, in contrast to treatment with chromium(III) compounds, which did not produce tumors (3, 14, 23). In rabbits, chromium(VI) also caused more damage in liver, kidney, and myocardium than did chromium(III) after an i.p. administration of potassium dichromate or chromium(III) nitrate (23). Chromium(VI) compounds have been shown to be more potent mutagens than chromium(III) compounds in the Salmonella typhimurium reversion assay (3).

Tissue levels of chromium in rats and mice have been measured after treatment with sodium chromate, chromium(III) nitrate, and chromium(III) chloride. After i.p. injections of either sodium chromate in mice or chromium(III) nitrate in rats, the highest levels of chromium were located in the kidney and liver (24, 28). The liver and kidney also accumulated large amounts of chromium following an i.v. injection of chromium(III) chloride (9, 27). Subcellular distribution of chromium has been measured in rat and mouse liver tissues (15, 24, 25). Chromium was concentrated in the nucleus following exposure to chromium(III) chloride but was more evenly distributed within the cell after exposure to sodium chromate (15, 24, 25).

Sodium dichromate caused DNA cross-links in rat liver, kidney, and lung nuclei; however, DNA damage was not observed in rat kidney 1 hr after injection of chromium(III) chloride (25). DNA cross-linking was generally correlated with nuclear chromium levels (25); however, it is not known whether this DNA damage is caused by direct interaction of the chromium with the DNA or by some indirect mechanism. Although isolation of chromium-DNA complexes formed in vivo has not been reported, in vitro studies have shown that stable complexes of chromium with DNA were formed after incubation of potassium dichromate with calf thymus DNA in the presence of microsomes and NADPH (26) and after incubation of chromium(III) chloride with DNA (2).

In order to explain the differences in the physiological effects of chromium(VI) and chromium(III) in vivo, it is necessary to determine the level of chromium bound to nucleic acids under conditions where the level of DNA damage is known. In this investigation, the in vivo interaction of chromium with chromatin and RNP3 was examined in rats following an i.p. injection of either sodium dichromate or chromium(III) chloride. Chromium was bound to DNA, nuclear proteins, and RNP in liver and kidney after exposure to both chromium(VI) and chromium(III). The kinetics of chromium binding and distribution in cellular fractions of the kidney and liver varied greatly between treatment with chromium(VI) and chromium(III). The ability of chromium(III) to cause DNA strand breaks and cross-links in rat tissues was also examined. No DNA damage was observed in kidney or liver after treatment with chromium(III) chloride.

MATERIALS AND METHODS

Chemicals. Sodium dichromate (Na2Cr2O7• 2H2O) and chromium(III) chloride (CrCl3• 6H2O) were purchased from Fisher Chemical Co., Pittsburgh, PA; proteinase K was purchased from Boehringer-Mannheim, Indianapolis, IN. All other chemicals were purchased from Sigma Chemical Co., St. Louis, MO, or Fisher Chemical Co.

Determination of Chromium. All buffers and reagents used for chromium analytical procedures were prepared "metal free" by treating them with sodium- equilibrated cation-exchange resin (AG 50W-X2; Bio-Rad, Richmond, CA). All glassware was pretreated with a solution of 1 g
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RESULTS

Natural Levels of Chromium on Chromatin and RNP. The distribution of chromium on chromatin and cytoplasmic RNP after injection of sodium dichromate and chromium(III) chloride is presented for liver and kidney in Table 1. In untreated rats, the level of chromium on liver chromatin was 7 ng chromium/g tissue and on RNP was 3 ng chromium/g tissue. The endogenous level of chromium was not statistically different (p > 0.05) on the kidney and liver chromatin but was 7 times greater (p < 0.0001) on the kidney RNP than on the liver RNP.

Distribution of Chromium on Liver Chromatin and RNP. In the liver, the highest level of chromium binding to chromatin and RNP occurred 4 hr after injection of sodium dichromate. After this time, the level of chromium bound to chromatin decreased through 40 hr. At 40 hr, approximately one-half of the maximal level of chromium remained bound to the chromatin. The level of chromium associated with chromatin after exposure to sodium dichromate was equal to the level of chromium on the RNP, except at 4 hr, where approximately one-half (p < 0.002) as much chromium was bound to the chromatin than to the RNP.

In contrast, following chromium(III) chloride treatment, the amount of chromium on the chromatin was 4 to 16 times greater (p < 0.05) than on the RNP. There was a general increase in chromium binding to chromatin and RNP through 40 hr (later times were not examined); however, a 5-fold increase in the level of chromium bound to RNP occurred between 24 and 40 hr after injection. Although approximately twice the amount of chromium was administered, the binding of chromium to chromatin after a 4-hr treatment with chromium(III) chloride was 4.5-fold lower (p < 0.0001) than after treatment with sodium dichromate. However, 12 hr after injection, there was no statistical difference in the level of chromium bound to chromatin (p > 0.05) for both chromium(III) chloride and sodium dichromate treatments. By 40 hr after injection, there was a 4-fold greater amount of chromium bound to chromatin following chromium(III) chloride treatment than after sodium dichromate treatment. At 4 to 24 hr after injection, there was 20 to 150 times more chromium bound to RNP after sodium dichromate treatment than after chromium(III) chloride treatment; however, by 40 hr after injection, the levels were the same (p > 0.05).

Distribution of Chromium on Kidney Chromatin and RNP. In the kidney, following sodium dichromate treatment, the amount of chromium bound to chromatin was equal to the amount of chromium bound to RNP, except at 4 hr, where the RNP had 3 times (p < 0.002) more chromium than did the chromatin. The highest level of chromium binding to chromatin was seen at 24 hr after injection. The levels of chromium bound to RNP was maximal 4 hr after treatment and decreased by 40 hr. Following chromium(III) chloride treatment, the chromatin had approximately one-half (p < 0.05) the amount of chromium bound compared to RNP, except at 24 hr. The level of chromium binding to chromatin increased with time, and leveled off by 24 hr. In contrast, there was a 4-fold increase in the level of chromium bound to RNP between 24 and 40 hr after chromium(III) chloride treatment. Although approximately twice the amount of chromium was administered, the binding of chromium to chromatin after treatment with chromium(III) chloride was 2 to 24 times lower (p < 0.01) than after treatment with sodium dichromate, except at 40 hr, where there was no significant difference (p >

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0.05) in the binding levels.

**Distribution of Chromium on Chromatin.** The interaction of chromium with liver DNA and chromosomal proteins varied dramatically between chromium(VI)- and chromium(III)-treated rats (Chart 1). In the liver, removal of the histone and nonhistone proteins and RNA from chromatin isolated after sodium dichromate treatment was accompanied by removal of 24 to 40% of the chromium bound to the chromatin (Chart 1A). The level of chromium binding to liver chromatin following chromium(III) chloride treatment decreased 80 to 87% after removal of histone and nonhistone proteins and RNA (Chart 1B). In the kidney, the level of chromium binding was decreased 26 to 57% after removal of histone and nonhistone proteins and RNA, following sodium dichromate treatment (Chart 2A). Detection of changes in the levels of chromium bound to DNA and protein fractions of kidney chromatin was more difficult after chromium(III) treatment, since the amount of chromium bound to chromatin was much lower than in the liver. However, 24 hr after injection, removal of the histone and nonhistone proteins and RNA removed 78% of the chromium bound to chromatin (Chart 2B).

**Binding of Chromium to Polynucleosomes and DNA.** The binding of chromium to the histone octamer proteins was examined for chromatin from liver and kidney (Chart 3). There was no significant difference (p > 0.05) between the chromium:nucleotide ratio of the DNA-histone octamer complex and the deproteinized DNA following chromium(III) chloride or sodium dichromate treatment in either liver or kidney, indicating that little or no chromium was bound to the histone octamer proteins.

In the liver, the binding of chromium to deproteinized DNA 4 to 24 hr after sodium dichromate treatment was 7 to 42 times (p < 0.02) greater than after chromium(III) chloride treatment, but at 40 hr, there was no significant difference in the binding levels. In the kidney, 5 to 8 times (p < 0.02) as much chromium was bound to the DNA at 4 to 12 hr after sodium dichromate treatment, as compared to chromium(III) chloride treatment. By 24 hr after chromium treatment, the levels were statistically the same (p > 0.05).

**Chromium(III) Chloride-mediated DNA Damage.** At 4, 24, and 40 hr after chromium(III) chloride injection, both liver and kidney tissues were assayed for DNA-protein cross-links, DNA interstrand cross-links, and DNA strand breaks. In contrast to the results with sodium dichromate (25), no damage was detected after chromium(III) chloride treatment by the alkaline elution method (Table 2).

**DISCUSSION**

The results of this in vivo distribution study have shown that chromium entered liver and kidney tissues at a slower rate after injection of chromium(III) chloride than after sodium dichromate.
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Chart 2. Chromium bound to rat kidney chromatin (•) and deproteinized DNA (A) after sodium dichromate (A), or chromium(III) chloride (8) treatment. Rats were treated as described in the legend to Chart 1. Points, mean; bars, S.E., determined for 4 rats; p < 0.04 versus control for all samples (19).

Chart 3. Chromium bound to rat liver (A) and kidney (B) DNA-histone octamer complex and deproteinized DNA after sodium dichromate and chromium(III) chloride treatment. Rats were treated as described in the legend to Chart 1. The amount of chromium (mol chromium/mol DNA-nucleotide) bound to the DNA-histone octamer complex [Cr(VI)] (A); [Cr(III)] (B) and deproteinized DNA [Cr(VI) (A); Cr(III) (C)] were calculated as described in "Materials and Methods." Points, mean; bars, S.E., determined for 4 rats; p < 0.05 versus control for all samples except for kidney deproteinized DNA samples 4 and 12 hr after chromium(III) treatment (19).

Table 2

<table>
<thead>
<tr>
<th>Exposure time (hr)</th>
<th>Tissue</th>
<th>Cross-links (rad equivalents)</th>
<th>Strand breaks (rad equivalents)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver (N = 3)</td>
<td>Chromium(III) chloride</td>
<td>Sodium dichromate*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Proteinase K</td>
<td>+Proteinase K</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>17.9 ± 14.1bc</td>
<td>32.4 ± 46.5</td>
</tr>
<tr>
<td>24</td>
<td>22.9 ± 25.7</td>
<td>6.0 ± 58.9</td>
<td>13 ± 3</td>
</tr>
<tr>
<td>36</td>
<td>4.1 ± 21.5</td>
<td>5.9 ± 24.5</td>
<td>7 ± 2</td>
</tr>
<tr>
<td>40</td>
<td>8.8 ± 20.5</td>
<td>35.1 ± 27.7</td>
<td>11 ± 6</td>
</tr>
<tr>
<td></td>
<td>Kidney (N = 2)</td>
<td>Chromium(III) chloride</td>
<td>Sodium dichromate*</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>-8.7 ± 16.5</td>
<td>29.9 ± 5.4c</td>
</tr>
<tr>
<td>24</td>
<td>3.7 ± 17.1</td>
<td>9.7 ± 20.1</td>
<td>13 ± 4c</td>
</tr>
<tr>
<td>40</td>
<td>6.7 ± 19.0</td>
<td>3.4 ± 2.2</td>
<td>7 ± 1c</td>
</tr>
</tbody>
</table>

* Data from Ref. 25.
bc Mean ± S.E.
cp < 0.05 versus control.
dp < 0.1 versus control.

However, chromium(III) did penetrate liver and kidney cells and was slowly bound to both RNP and chromatin. Other in vivo studies have demonstrated that chromium entered liver and kidney cells of rodents after i.p. and i.v. injection of chromium(III) chloride and chromium(III) nitrate (15, 20, 24). In contrast, in in vitro cell systems, such as RBC (8), rat intestinal cells (6), and Ehrlich mouse ascites carcinoma cells (18), no uptake of chromium(III) was observed, suggesting that in vitro cell membranes are not permeable to simple chromium(III) compounds. Chromium(VI) was readily taken up in these same cell systems. Since the oxidation of chromium(III) to chromium(VI) is unlikely in biological systems (17), the slow uptake of chromium(III) by liver and kidney tissues in vivo is probably due to the slow formation of a biological complex of chromium(III) which is able to penetrate cell membranes.

Analysis of chromatin and RNP from livers of rats treated with chromium(III) chloride revealed that chromium was preferentially localized on the chromatin. Nuclear localization of the chromium was not seen in the kidney, but less chromium(III) entered kidney cells than liver cells. Other subcellular distribution studies in liver...
tissue showed that chromium was concentrated in the nucleus following an i.p. injection of either chromium(III) chloride in rats (15) or chromium(III) nitrate in mice (24). Copper, zinc, and manganese did not concentrate in the nucleus of rat liver after administration of the metal salts in the diet (16).

After chromium(VI) treatment, thechromium was located on both the chromatin and RNP fractions in the liver and kidney. The amount of chromium bound to chromatin plateaued at 12 hr in the kidney but decreased after 4 hr in the liver. In contrast, after chromium(III) treatment, there was an increase in chromium binding to chromatin in the liver through 40 hr. The rapid accumulation of chromium in rat liver nuclei following sodium chromate treatment, and the slower but continuous increase of chromium after chromium(III) chloride treatment, was also seen after an i.v. injection of the chromium compounds (20). The large increase in the level of chromium bound to liver and kidney RNP between 24 and 40 hr after injection of chromium(III) chloride was not observed in the chromatin samples. It is possible that chromium(III) induces a cytoplasmic factor which influences the uptake and subsequent binding of chromium(III) to RNP.

Previously, Tsapakos et al. (25) reported a correlation between DNA cross-link damage and nuclear levels of chromium following an i.p. injection of sodium dichromate, and suggested that chromium may cause DNA damage through a direct interaction with the chromatin. In the liver, maximal DNA-protein cross-linking (25) and maximal chromium binding to chromatin (Chart 1) were seen at 4 hr after injection of chromium. Although no DNA cross-links (25) were detected 36 hr after treatment, some chromium was still bound to the chromatin. In the kidney, the binding of chromium to chromatin increased up to 12 hr and then leveled off through 40 hr (Chart 2), but the DNA-protein cross-links were maximal from 1 to 8 hr and then decreased through 40 hr (25). It appears that there is no direct correlation between chromium binding to chromatin and DNA cross-links.

Apparently, none of the chromium(III) complexes with chromatin were able to cause detectable DNA-protein cross-links, DNA interstrand cross-links, or DNA strand breaks (Table 2). This again suggests that chromium binding to the chromatin is not sufficient to cause these DNA lesions. It may also be necessary for the chromium to be directly bound to the DNA. After chromium(III) treatment, very little of the chromium associated with chromatin was actually bound to the DNA (Chart 3). In contrast, following chromium(VI) treatment, a larger portion of the chromium associated with the chromatin was bound to the DNA. However, at 40 hr after either sodium dichromate or chromium(III) chloride exposure (Chart 3), there were detectable levels of chromium bound to the deproteinized DNA in both the liver and kidney, but measurable levels of DNA protein cross-links were present only in the kidney of rats treated with sodium dichromate. It is possible that other types of DNA damage not detectable by alkaline elution, such as DNA intrastrand crosslinks or monooadducts with the bases, could be formed following chromium(III) or chromium(VI) treatments. It is possible that chromium(III) will cause detectable DNA damage at longer times. However, the lack of mutagenicity of chromium(III) chloride, along with chromium(VI) being a potent mutagen in the S. typhimurium assay (3), suggests that DNA damage is less likely following chromium(III) than chromium(VI) treatment.

It is likely that multiple chromium-DNA adducts will be formed after treatment with chromium(III) chloride and after treatment with sodium dichromate and that the nature of the adducts will differ, depending on the oxidation state of the chromium compound administered. There is evidence for the in vitro binding of chromium(III) to the phosphate groups and guanine and cytosine bases of DNA (2, 22). The interaction of chromium with the phosphate groups is believed to be electrostatic. Preferential binding of chromium to guanine has been observed upon incubation of chromium(VI) with homopolyribonucleotides in the presence of a microsomal-metabolizing system (26). The nature of the chromium:guanine interaction has not been determined; however, binding of N-7, O-6, N-1, or N-2 are possibilities. Our data suggest that only certain types of chromium:DNA complexes formed after administration of chromium(VI) produce lesions in the DNA which are detectable by alkaline elution. The persistent DNA:protein cross-links observed after chromium(VI) treatment may arise from chromium:DNA adducts which are not recognized by DNA repair systems.

In conclusion, chromium was bound to DNA, nuclear proteins, and RNP in liver and kidney tissues, following an i.p. injection of sodium dichromate and chromium(III) chloride. At early times, there was much less chromium binding to chromatin and DNA after chromium(III) treatment than after chromium(VI) treatment. In addition, after chromium(III) treatment, a large percentage of the chromium bound to chromatin was associated with protein rather than with the DNA. However, 40 hr after injection there was no significant difference in the level of chromium binding to DNA after either chromium(VI) or chromium(III) treatment. At this time, DNA damage was found in the kidney only after chromium(VI) treatment, suggesting that not all types of chromium:DNA complexes produce DNA lesions.

REFERENCES


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