Chromium(VI)-induced DNA Lesions and Chromium Distribution in Rat Kidney, Liver, and Lung

Michael J. Tsapakos, Thomas H. Hampton, and Karen E. Wetterhahn

ABSTRACT

DNA lesions were detected in rat organ nuclei following an i.p. injection of sodium dichromate. Kidney, liver, and lung nuclei were examined for DNA interstrand cross-links, strand breaks, and DNA-protein cross-links using the alkaline elution technique. The time course for formation of cross-links in kidney nuclei revealed the presence of DNA interstrand and DNA-protein cross-links 1 hr after injection of sodium dichromate. By 40 hr in kidney, DNA interstrand cross-links had been repaired, but DNA-protein cross-links persisted. In liver nuclei, the time course for formation of cross-links after injection of dichromate showed a maximum in DNA-protein cross-linking at 4 hr and a maximum in DNA interstrand cross-linking at 2 hr. By 36 hr, in the liver, both types of lesions had been repaired. In lung nuclei, both DNA interstrand and DNA-protein cross-links were observed 1 hr after dichromate injection; however, by 36 hr, only DNA-protein cross-links persisted. No DNA lesions were detectable in kidney 1 hr after an i.p. injection of chromium(III) chloride. Chromium distribution in rat kidney, liver, and lung was measured and is discussed with respect to the observed DNA lesions. The lung and kidney may be more sensitive than liver to chromium-induced DNA damage, an observation which correlates with the reported toxicity and carcinogenicity data for chromium(VI) in both animals and humans.

INTRODUCTION

Chromium(VI) compounds pose a serious occupational health hazard in terms of both their potential respiratory tract carcinogenicity and their toxic effect on other organs. Extensive epidemiological evidence has been published on the high incidence of respiratory tract cancer in chromate workers in various countries (7, 25). In addition, renal and hepatic damage resulting from exposure to chromium(VI) compounds has been reported for chromium workers (24). In animals, several studies have reported the induction of tumors by chromium(VI) compounds at the injection and implantation site (24, 25). In dust inhalation studies with animals, chromium(VI) compounds were reported as lung carcinogens and as remote-site carcinogens (24). In addition, renal (1-3, 15-17, 19, 23, 26, 30-32) and hepatic (32) damage has resulted in animals after injection of chromium(VI) compounds by various routes.

The interaction of chromium compounds with nucleic acids is important to the mechanism of chromium(VI)-induced carcinogenicity. In vitro, the presence of a rat liver microsomal metabolizing system was necessary in order to observe reaction between chromium(VI) and DNA (or RNA) (35). These studies suggested that chromium formed a stable ternary complex with DNA and protein after metabolism of chromium(VI) to chromium(III). Using the alkaline elution technique, DNA-protein cross-links have been observed upon treatment of various cultured mammalian cells with chromium(VI) compounds (4, 14, 22). These DNA-protein cross-links have been observed in cultured cells treated with chromium(VI) (4, 13, 14).

The alkaline elution technique (13, 14, 22) and alkaline sucrose gradients (8, 28, 36) have been used to detect DNA strand breaks in cultured mammalian cells treated with chromium(VI) compounds. In studies where the toxicity of chromium(VI) was measured, DNA strand breaks were observed only at chromium(VI) concentrations which were ~100-fold higher than those which produced a detectable toxic effect on the cells (8, 28, 36). Other studies reported that no DNA strand breaks were observed by alkaline elution (4) or by alkaline sucrose gradient (5, 8) analysis of cultured cells treated with chromium(VI) compounds. Repair of chromium(VI)-induced DNA strand breaks was observed in human embryonic lung fibroblasts (IMR-90 cells) 4 or 12 hr after removal of chromium(VI) (14). Unscheduled DNA synthesis was observed in cultured human skin fibroblasts (36) and mouse fetal cells (27) exposed to chromium(VI) compounds. An increase in the amount of DNA strand breakage was observed in the presence of a DNA polymerase inhibitor in chromium(VI)-treated normal and xeroderma pigmentosum human fibroblasts (13). It was concluded that chromium(VI)-induced DNA damage must be repaired by a system other than the UV excision repair system (13).

Since metabolism of chromium(VI) appears to be important to the interaction of chromium with DNA and since chromium(VI) induced variable DNA damage and repair in different cultured cell systems, we have studied in vivo DNA damage in various tissues of rats treated with chromium(VI). We previously reported that DNA damage occurred in vivo in rat kidney and liver 1 hr after an i.p. injection of sodium dichromate (34). The present study describes the time course for formation and removal of DNA lesions in rat kidney, liver, and lung over a period of 0 to 40 hr after an i.p. injection of sodium dichromate. The distribution of chromium in the tissues and in the nuclei isolated from these tissues was measured 0 to 24 hr after injection using [51Cr]-chromate. The chromium distribution in these organs is discussed with respect to the observed DNA damage.

MATERIALS AND METHODS

Chemicals. Sodium dichromate (Na2Cr2O7· 2H2O) and chromic chloride (CrCl3· 6H2O) were purchased from Fisher Chemical Co., Pittsburgh, Pa.: 51Cr, as Na51CrO4 in 0.9% NaCl solution, 2.5 μg chromium per ml, 1 mCi per ml, was purchased from Amersham, Inc., Arlington Heights.

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Chromium(VI)-induced DNA Lesions

Chromate. Male Sprague-Dawley [CRL:CD(SD)BR] rats (Charles River Breeding Labs) weighing 150 to 200 g were given i.p. injections of 0.5 ml of 0.9% NaCl solutions containing appropriate doses of cold Na$_2$Cr$_2$O$_7$. Typically, $30$ to $50$ μCi of $^{51}$Cr were administered per rat in addition to the appropriate dose of cold Na$_2$Cr$_2$O$_7$. One g of lung, liver, and kidney tissue was removed and homogenized, as specified above for the nuclei preparation, and counted on a Beckman Model 8000 gamma counter (Beckman Instruments, Inc., Fullerton, Calif.). Sucrose-purified nuclei, prepared as described above, were also counted for $^{51}$Cr. After subtracting background cpm and correcting cpm for decay during the course of the experiment, the known specific activity of the injected chromate solution was used to calculate ng chromium per g tissue and per $10^6$ nuclei.

RESULTS

Chromate-induced DNA Damage in Rat Tissues. DNA damage in rat kidney, liver, and lung following an i.p. injection of sodium dichromate was measured by the alkaline elution technique on nuclei isolated from these organs. DNA strand breaks, DNA-protein cross-links, and interstrand cross-linking were evaluated as specified by Kohn et al. (20).

The time course for formation of DNA cross-links in rat kidney after injection of sodium dichromate (20 mg/kg body weight) is presented in Chart 1. Within 1 hr after injection, total cross-linking reached a maximum. At 12 hr, total cross-linking decreased to a lower but significant level which persisted to 40 hr (longer times after injection were not examined). In contrast with the decreasing trend in cross-linking observed at the 20-mg/kg dose of sodium dichromate between 4 and 12 hr (Chart 1), the level of cross-linking increased between 4 and 12 hr at the 40-mg/kg dose (Table 1). Upon treatment of the kidney nuclear lysates with proteinase K, DNA-protein cross-links were removed revealing the presence of interstrand cross-links. Interstrand cross-linking was observed at a constant level 1 through 24 hr after injection of 20 mg/kg sodium dichromate (Chart 1); however, by 40 hr, the interstrand cross-links were removed. Interstrand cross-linking was also seen at both 4 and 12 hr after injection of the 40 mg/kg dose (Table 1). No cross-linking was observed in the kidney 1 hr after injection of chromium(III) chloride at 80 mg/kg, a dose

### Table 1

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Compound</th>
<th>Dose (mg/kg)</th>
<th>Exposure (hr)</th>
<th>Without proteinase K</th>
<th>With proteinase K</th>
<th>Without proteinase K</th>
<th>With proteinase K</th>
<th>Cross-link coefficient (rad equivalents)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>Na$_2$Cr$_2$O$_7$-2H$_2$O</td>
<td>20</td>
<td>4</td>
<td>$10 \pm 4^a$ (3)</td>
<td>$11 \pm 3^a$ (6)</td>
<td>$0.128 \pm 0.009^b$ (5)</td>
<td>$0.039 \pm 0.006^b$ (3)</td>
<td>0.128 \pm 0.009^b (5)</td>
</tr>
<tr>
<td></td>
<td>CrCl$_3$-6H$_2$O</td>
<td>80</td>
<td>1</td>
<td>$0 \pm 1^a$ (3)</td>
<td>$0 \pm 1^a$ (3)</td>
<td>$0.076 \pm 0.013^b$ (3)</td>
<td>$0.027 \pm 0.003^b$ (3)</td>
<td>0.076 \pm 0.013^b (3)</td>
</tr>
<tr>
<td>Liver</td>
<td>Na$_2$Cr$_2$O$_7$-2H$_2$O</td>
<td>20</td>
<td>4</td>
<td>$11 \pm 6^a$ (6)</td>
<td>$-6 \pm 2^a$ (3)</td>
<td>$0.289 \pm 0.046^b$ (6)</td>
<td>$0.027 \pm 0.006^b$ (3)</td>
<td>0.289 \pm 0.046^b (6)</td>
</tr>
<tr>
<td></td>
<td>CrCl$_3$-6H$_2$O</td>
<td>80</td>
<td>1</td>
<td>$0 \pm 1^a$ (3)</td>
<td>$15 \pm 5^a$ (3)</td>
<td>$0.073 \pm 0.006^b$ (5)</td>
<td>$-0.005 \pm 0.003^b$ (3)</td>
<td>0.073 \pm 0.006^b (5)</td>
</tr>
<tr>
<td>Lung</td>
<td>Na$_2$Cr$_2$O$_7$-2H$_2$O</td>
<td>20</td>
<td>1</td>
<td>$7 \pm 4^a$ (4)</td>
<td>$11 \pm 3^a$ (3)</td>
<td>$0.015 \pm 0.016^b$ (2)</td>
<td>$0.001 \pm 0.006^b$ (3)</td>
<td>0.015 \pm 0.016^b (2)</td>
</tr>
<tr>
<td></td>
<td>CrCl$_3$-6H$_2$O</td>
<td>80</td>
<td>1</td>
<td>$28 \pm 5^a$ (3)</td>
<td>$-62 \pm 5^a$ (3)</td>
<td>$-0.022 \pm 0.004^b$ (3)</td>
<td>$-0.008 \pm 0.004^b$ (3)</td>
<td>-0.022 \pm 0.004^b (3)</td>
</tr>
</tbody>
</table>

Mean ± S.E. Calculated by the equations of Ewig and Kohn (12). p < 0.01 versus control, t test (36).

Numbers in parentheses, number of rats tested.

p < 0.01 versus control, t test (36).

p > 0.05 versus control, t test (36).

p > 0.1 versus control, t test (36).
which is equimolar in chromium to a 45-mg/kg dose of sodium dichromate (Table 1).

The time course for induction of cross-links in rat liver as a function of time after injection of sodium dichromate (20 mg/kg) is presented in Chart 2. In contrast to kidney, total cross-links in liver rose sharply to a peak at 4 hr after injection and then declined rapidly through 8 hr. The maximum amount of total cross-linking in the liver was approximately twice that observed in the kidney (Table 1). However, unlike the kidney, cross-linking had completely disappeared in the liver 36 hr after injection. The interstrand cross-linking in the liver reached a maximum value at 2 hr after injection but had disappeared by 8 hr. Total cross-linking in liver nuclear DNA at the higher dose of sodium dichromate (40 mg/kg) remained at approximately the same level at 4 and 12 hr after injection (Table 1) in contrast to the decrease in cross-linking seen between 4 and 12 hr after injection at the lower dose (20 mg/kg). At the higher dose, no interstrand cross-linking was observed at 4 hr, and only a low level of interstrand cross-linking was detected 12 hr after injection.

The time course for formation of cross-links in lung after injection of sodium dichromate is presented in Chart 3. As in both liver and kidney, significant cross-linking in the lung was observed 1 hr after injection, and total cross-linking peaked at 4 hr. Little or no cross-linking was observed in lung 1 or 4 hr after injection of the lower dose (20 mg/kg) (Table 1). By 24 hr after injection of sodium dichromate (40 mg/kg), total cross-linking had decreased to about one-half the maximum value, and this level persisted through 36 hr. Interstrand cross-linking in the lung reached maximum 1 hr after injection and gradually declined to 0 after 36 hr. Thus, as with kidney, DNA-protein cross-linking was the major cross-linking lesion to persist through 36 hr in the lung.

Only low levels of DNA strand breaks (in the presence or absence of proteinase K treatment) were observed in rat kidney and liver at various times after injection of sodium dichromate (20 or 40 mg/kg) (Table 1 and data not shown). No strand breaks in rat lung were observed in the absence of proteinase K digestion through 24 hr; however, a small amount of strand breakage [24 ± 3 (S.E.) rad equivalent] was observed 36 hr after injection of sodium dichromate (40 mg/kg) (Table 1 and data not shown). Upon proteinase K treatment of the lysates, DNA strand breaks in rat lung (maximum of 28 ± 6 rad equivalents) appeared at 4 to 12 hr after injection of sodium dichromate (40 mg/kg), were removed, and then reappeared at 36 hr (33 ± 3 rad equivalents) (Table 1 and data not shown). Only low levels of strand breaks were observed in rat lung after injection of sodium dichromate (20 mg/kg) (Table 1). At the 20-mg/kg dose, a maximum of 21 ± 3 rad equivalents of strand breaks were observed in rat liver 1 hr after injection; however, no strand breaks remained by 24 or 36 hr (data not shown). In the kidney, DNA strand breaks (12 ± 6 rad equivalents maximum) were observed 4 to 8 hr after injection of 20 mg/kg; however, all strand breaks were removed by 12 hr (Table 1), and then strand breaks were observed only after proteinase K treatment at 24 (9 ± 1 rad equivalents) and 40 hr (14 ± 2 rad equivalents) (data not shown). No strand breaks were observed in rat kidney 1 hr after injection of chromium(III) chloride (80 mg/kg) (Table 1).

**Chromium Distribution.** The concentration of chromium in rat kidney, liver, and lung tissues was measured after a single 20- or 40-mg/kg injection of Na₂Cr₂O₇ containing Na₂⁵¹CrO₄ at a known specific activity. At the 40-mg/kg dose, levels of chromium for liver tissue (3.18 ± 0.33% dose administered per g tissue) and kidney tissue (3.41 ± 0.29% dose administered per g tissue) peaked 12 hr after injection (Chart 4). In kidney and liver tissue at the 20-mg/kg dose, levels of chromium reached maximum at 4 hr after injection (2.42 ± 0.40% dose administered per g kidney tissue; 2.91 ± 0.68% dose administered per g liver tissue) (Chart 4). A significant amount of chromium was in the tissues by 1 hr (1.73 ± 1.16% dose administered per g kidney tissue; 1.77 ± 0.74% dose administered per g liver tissue). At a 40-mg/kg dose of sodium dichromate for both kidney and liver, chromium levels in nuclei increased with time after injection.
At 12 to 24 hr after injection, the chromium levels in liver nuclei were higher than they were in kidney nuclei. At the lower (20-mg/kg) dose of sodium dichromate, the amount of chromium in both kidney and liver nuclei reached a maximum at 4 hr after injection and decreased to a low but persistent level at 8 through 24 hr (Chart 5).

Chromium distributions in lung tissue and nuclei are presented in Chart 6. Generally, the levels of chromium in the lung were 5 to 10 times lower than those found in kidney and liver. At a 40-mg/kg dose of sodium dichromate, as with liver and kidney, the lung tissue chromium concentration was maximum 12 hr after injection (0.61 ± 0.10% dose administered per g tissue) (Chart 6A). The lower dose of 20 mg/kg produced maximum chromium concentration in lung tissue 1 hr after injection (0.27 ± 0.22% dose administered per g tissue). Lung nuclei chromium levels (Chart 6B) generally followed the same trends which were observed in the lung tissue with both doses of sodium dichromate. At the 20-mg/kg dose of sodium dichromate, chromium levels in lung nuclei peaked 4 hr after injection, decreased through 8 hr, and then increased slightly through 24 hr. At the higher (40-mg/kg) dose, chromium levels in lung nuclei peaked at 12 hr after injection followed by a decline through 24 hr.

The number of nuclei which sedimented through sucrose in the chromium distribution study remained fairly constant regardless of the source of nuclei and time after injection of sodium dichromate, indicating that minimal lethal cell damage had occurred in any of the organs analyzed under any of the conditions used in our experiments.

**DISCUSSION**

We reported previously that chromium(VI) induced DNA-protein cross-links and low levels of DNA strand breakage in rat liver and kidney 1 hr after i.p. injection of sodium dichromate (34). The time course for formation and removal of DNA strand breaks, DNA-protein cross-links, and interstrand cross-links has been presented for kidney, liver, and lung after exposure of rats to sodium dichromate. Chromium(VI) induced significant levels of DNA-protein and interstrand cross-linking in all 3 organs within the first 4 hr after injection. The major form of cross-links was DNA-protein cross-links. Other studies (4, 14, 22) of chromium(VI)-induced DNA damage in cultured mammalian cells reported the occurrence of DNA-protein cross-links but not interstrand cross-links. Formation of DNA-protein cross-links in rat kidney and lung appeared to saturate and then gradually declined to a lower but persistent level. The level of interstrand cross-links in rat kidney and lung remained fairly constant between 1 and 12 to 24 hr after injection, but in contrast to DNA-protein cross-links, the interstrand cross-links were not persistent. These results may indicate that a steady state situation exists between formation and removal of interstrand and DNA-protein cross-links which directly involve chromium. It is possible that the persistent DNA-protein cross-links result from cross-linking between DNA and repair enzymes which were activated to repair DNA lesions induced by chromium(VI). The persistence of some of the DNA-protein cross-links may indicate that in kidney and lung this DNA lesion is not recognized by cellular repair proteins or that the various cell types in kidney and lung have different capacities to repair the chromium(VI)-induced DNA-protein cross-links. A recent study showed that in vivo repair, as measured by unscheduled DNA synthesis in mouse skin treated with various chemical carcinogens, was 3- to 5-fold more active in the mouse epithelial cells than in the dermal fibroblasts (18).

The trends in the cross-linking time courses for kidney and lung can be contrasted with that for liver after exposure to chromium(VI). In liver, DNA-protein cross-links are formed within 4 hr; however, 90% were removed by 8 hr, and no cross-links persisted in this organ. It also appears that interstrand cross-links are repaired faster in liver than in kidney and lung, since in liver interstrand cross-links were completely removed 8 hr following injection. These results may indicate that rat liver cells, which possess high metabolic activity, may be more efficient at repairing chromium(VI)-induced DNA damage than are kidney or lung cells.

The results of the chromium distribution studies indicate that significant levels of chromium accumulate in rat kidney, liver, and lung at relatively short times following an i.p. injection of sodium dichromate spiked with radioactive chromium(VI). These findings confirm results of other studies of chromium distribution in rats after i.v. administration of sodium chromate (21, 29) and s.c. administration of potassium dichromate (37). The higher chromium levels were found in the liver and kidney, with the liver having the highest levels at all times for both doses of chromium(VI) examined. Levels of chromium in lung tissue were approximately 10 to 20% of those observed in liver at all times for both doses. The very low level of chromium distributed to the lung tissue and nuclei after the 20-mg/kg dose probably accounts...
for the lack of observable DNA damage under these conditions.

The trends in chromium levels in rat liver and kidney nuclei varied depending on the dose that was administered. DNA damage in the form of DNA cross-linking generally correlated with the chromium levels in liver and kidney nuclei. Maximum damage as DNA-protein cross-linking in liver nuclei occurred at 4 hr after injection of sodium dichromate (20 mg/kg), correlating well with the maximum nuclear chromium level at this time and dose. In kidney nuclei at this dose, the correlation was less clear, since chromium levels dropped after 4 hr; yet DNA-protein cross-linking persisted at a high level through 8 hr. By 24 hr, cross-linking had been significantly reduced in both liver and kidney, correlating with a decrease in nuclear chromium levels at this time and dose. In both kidney and liver, DNA cross-links and nuclear chromium levels were higher at the lower dose (20 mg/kg) than at the higher dose (40 mg/kg) 4 hr after injection. However, by 12 hr after injection DNA cross-links and nuclear chromium levels were higher at the 40-mg/kg dose than at the lower dose. In contrast to the decrease in nuclear liver and kidney chromium levels after 4 hr at the 20-mg/kg dose of sodium dichromate, there is a steady increase in chromium levels with time after exposure to 40 mg/kg in these organs. In kidney, this increase correlates with increased DNA-protein and interstrand cross-linking observed between 4 and 12 hr after injection. In liver nuclei, which were found to contain much higher levels of chromium than kidney nuclei 12 hr after injection, DNA-protein cross-linking reached a plateau at 4 through 12 hr. It is possible that repair mechanisms which are functional in the liver at the lower dose may be either inhibited or saturated at the higher dose. Chromium may be interacting with nuclear proteins, such as repair enzymes, either inhibiting or blocking their function.

It appears that lung and kidney are more sensitive than liver to the low levels of chromium in the nucleus after injection of sodium dichromate. Lung nuclear chromium levels at saturation of DNA-protein cross-linking (approximately 10 to 17 ng chromium per 10^6 nuclei) were close to kidney nuclear chromium levels at saturation of cross-linking (approximately 15 to 25 ng chromium per 10^6 nuclei). However, the corresponding value for liver was much higher (approximately 60 to 70 ng chromium per 10^6 nuclei). In contrast to chromium(VI), chromium(III) produced no detectable strand breaks or cross-links in the rat kidney 1 hr after injection. Other studies have shown that after s.c. injection of chromium(III) in rats, 10- to 100-fold lower levels of chromium are detected in kidney, liver, and lung tissues than after injection of chromium(VI) at a dose equimolar in chromium (37). In addition, lower levels of chromium were detected in liver nuclei up to 12 hr after i.v. injection of chromium(III) compared to an equimolar injection of chromium(VI) (29). These results correlate with the greater kidney and liver toxicity of chromium(VI) compared to chromium(III) after i.p. injection in rabbits (23, 32). No DNA cross-links were observed in human lung fibroblasts or mouse L1210 leukemia cells treated with potassium dichromate. Proc. Am. Assoc. Cancer Res., 21: 98, 1980.


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