DNA Single-strand Breaks and Cytotoxicity Induced by Chromate(VI), Cadmium(II), and Mercury(II) in Hydrogen Peroxide-resistant Cell Lines

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The induction of cytotoxicity and DNA single-strand breaks by chromium(VI), cadmium(II), and mercury(II) have been investigated in CHO resistant Chinese hamster ovary (CHO<sup>R</sup>) cells and parental (CHO<sup>P</sup>) cells. Using a colony-forming assay, CHO<sup>R</sup> cells were found to be significantly more resistant than CHO<sup>P</sup> cells to the cytotoxicity caused by CdCl<sub>2</sub> and HgCl<sub>2</sub>, but not to that caused by Na<sub>2</sub>CrO<sub>4</sub>. However, the DNA single-strand breaks produced by each of these metals were significantly lower in the CHO<sup>R</sup> cells. With respect to chromium reduction, the level of chromium(V) in CHO<sup>R</sup> cells was decreased. The role of intracellular active oxygen in the heavy metal-induced DNA damage and cytotoxicity is discussed. — Environ Health Perspect 102(Suppl 3):341-342 (1994).

Key words: chromate(VI), cadmium(II), mercury(II), hydrogen peroxide, active oxygen

**Introduction**

Toxic and carcinogenic heavy-metal compounds, such as chromium(VI), cadmium(II), and mercury(II), have been shown to produce DNA single-strand breaks in cultured mammalian cells (1-6). Previous studies have demonstrated that these metal compounds may cause adverse biologic effects, possibly through free radical species such as active oxygen, because active oxygen scavengers prevented metals-induced cellular injuries such as DNA breaks (4-6). With respect to chromium(VI), although active oxygen species have been reported to be generated during reduction of chromium(VI) to chromium(V) in vitro (3,7-11), only one study examined the involvement of active oxygen species in chromium(VI)-induced genotoxicity and cytotoxicity in intact cells (6). In addition, the relationship between active oxygen and cytotoxicity of mercury(II) and of cadmium(II) is not clear. In the present study, using hydrogen peroxide-resistant cell lines, we investigated the DNA breaks and cytotoxicity produced by these heavy metals to elucidate the role of active oxygen in the mechanism of their toxic effects (12).

**Materials and Methods**

Hydrogen peroxide-resistant Chinese hamster ovary (CHO<sup>R</sup>) cells were derived from the parental (CHO<sup>P</sup>) cells by adding progressively higher concentrations (6.25 to 100 μM) of H<sub>2</sub>O<sub>2</sub> in stepwise fashion to the culture medium (12). Logarithmically growing cells were treated with H<sub>2</sub>O<sub>2</sub> for 1 hr or with Na<sub>2</sub>CrO<sub>4</sub>, CdCl<sub>2</sub>, and HgCl<sub>2</sub> for 2 hr in saline-glucose medium (50 mM Hepes, pH 7.2) containing 10 mM NaCl, 5 mM KCl, 2 mM CaCl<sub>2</sub>, and 5 mM glucose. DNA single-strand breaks and cytotoxicity were estimated by alkaline elution assay and colony forming assay, respectively (13-15). To detect chromium(V) in cells, the harvested cells were mixed with 400 μM Na<sub>2</sub>CrO<sub>4</sub>, and then placed in an electronic spin resonance (ESR) tube. ESR measurements were carried out during the incubation at room temperature (12).

**Results**

Table 1 shows that the concentration of H<sub>2</sub>O<sub>2</sub> that reduced colony formation by 50% (ID<sub>50</sub>) was 5-fold less in CHO<sup>R</sup> cells than in CHO<sup>P</sup> cells. CHO<sup>R</sup> cells were also cross-resistant to CdCl<sub>2</sub> and HgCl<sub>2</sub>, but not to Na<sub>2</sub>CrO<sub>4</sub>.

Table 2 shows the induction of DNA single-strand breaks produced by H<sub>2</sub>O<sub>2</sub> or the metal compared in CHO<sup>R</sup> and CHO<sup>P</sup> cells. The formation of DNA breaks induced by H<sub>2</sub>O<sub>2</sub> was almost completely inhibited in CHO<sup>R</sup> cells. Although the cytotoxicity was not affected, the formation of DNA breakage produced by Na<sub>2</sub>CrO<sub>4</sub> was significantly reduced by about 15% in CHO<sup>R</sup> cells compared to that in CHO<sup>P</sup> cells. CHO<sup>R</sup> were also cross-resistant to DNA breaks caused by CdCl<sub>2</sub> and HgCl<sub>2</sub>.

It is unlikely that the observed metal-resistance in CHO<sup>R</sup> cells was due to alterations of metal uptake, because there was no difference in the uptake of the metals in these cell lines (12). Since chromium(VI) enters cells and is then reduced to chromium(III), the formation of chromium(III) and other oxidation states, in particular chromium(V), is believed to play a role in some of the biological effects of chromium(VI).}

**Table 1.** Hydrogen peroxide and metal sensitivity of CHO<sup>P</sup> and CHO<sup>R</sup> cells.

| Treatment | CHO<sup>P</sup> (mM) | CHO<sup>R</sup> (mM) |
|-----------|----------------|-------------------|
| H<sub>2</sub>O<sub>2</sub> | 34 | 175 |
| Na<sub>2</sub>CrO<sub>4</sub> | 15.5 | 14.7 |
| CdCl<sub>2</sub> | 6.1 | 1.6 |
| HgCl<sub>2</sub> | 0.88 | 0.26 |

*ID<sub>50</sub> values are the concentrations that inhibit colony formation by 50% after treatment with H<sub>2</sub>O<sub>2</sub> for 1 hr, or with the metals for 2 hr.*
chromium(VI) (1–3). In fact, relatively long-lived chromium(VI) species formed during reduction of chromium(VI) have been shown to induce DNA breaks by the generation of active oxygen radicals in vitro (7–10). For this reason, we also examined the formation of chromium(V) in both cell lines. The results showed the appearance of the chromium(V) ESR signal with a maximum peaks at g = 1.978 during the incubation of cells with Na$_2$CrO$_4$ and it was found that the level of this chromium(V) was about 2-fold lower in the resistant cells (12).

### Discussion

Previous studies have reported that the induction by cadmium(II) and mercury(II) of damages such as DNA breaks is mediated by the formation of active oxygen species in cultured cells (4–6). The present results indicate that both the cytotoxicity and the DNA breakage induced by these two metals may be associated with active oxygen species inside cells, because CHO$^R$ cells were resistant only to DNA breaks, but not to the cytotoxicity caused by Na$_2$CrO$_4$, indicating that active oxygen species inside cells may partly associated with chromium(VI)-induced DNA strand breaks. Since the levels of chromium(V) in CHO$^R$ cells were lower than those in CHO$^S$ cells, chromium(V) may also participate in the induction of DNA breaks. The relationship between active oxygen species and chromium(V) formation in cells is under investigation.

### Conclusion

We examined the induction of cytotoxicity and DNA breaks by chromate(VI), cadmium(II), and mercury(II) in hydrogen peroxide-resistant cells. Cytotoxicity was not affected; however, a cross-resistance was found against DNA breaks caused by sodium chromate(VI). Cross-resistance was observed with respect not only to DNA breaks but also to the cytotoxicity produced by cadmium(II) and mercury(II). Hydrogen peroxide-resistant cells may be useful for the examination of involvement of active oxygen species induced by toxic metal compounds.

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**Table 2. Induction of DNA single-strand breaks induced by hydrogen peroxide and metal compounds.**

| Treatment $^a$ | CHO$^S$ ($\mu$M) | CHO$^R$ ($\mu$M) |
|---------------|------------------|------------------|
| H$_2$O$_2$    | 25               | 0.63 ± 0.05$^b$ | 0.05 ± 0.006$^c$ |
| Na$_2$CrO$_4$ | 400              | 0.50 ± 0.06$^d$ | 0.28 ± 0.068$^e$ |
| CdCl$_2$     | 300              | 0.49 ± 0.030     | 0.04 ± 0.019$^f$ |
| HgCl$_2$     | 2                | 0.510 ± 0.082    | 0.164 ± 0.096$^g$ |

$^a$Cells were treated with H$_2$O$_2$ for 1 hr or with the metals for 2 hr and then DNA breaks were measured as described (12,14). $^b$Each value is the mean ± SD. *p<0.01 (Student’s t-test).