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

Katsuyuki Tsuzuki, Masayasu Sugiyama, and Nobuya Haramaki

Department of Medical Biochemistry, Kurume University School of Medicine, Kurume, Japan

The induction of cytotoxicity and DNA single-strand breaks by chromium(VI), cadmium(II), and mercury(II) were compared in H_2O_2-resistant Chinese hamster ovary (CHO^R) cells and parental (CHO^P) cells. Using a colony-forming assay, CHO^R cells were found to be significantly more resistant than CHO^P cells to the cytotoxicity caused by $CdCl_2$ and $HgCl_2$, but not to that caused by Na_2CrO_4. However, the DNA single-strand breaks produced by each of these metals were significantly lower in the CHO^R cells. With respect to chromium reduction, the level of chromium(V) in CHO^R 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*).

This paper was presented at the Second International Meeting on Molecular Mechanisms of Metal Toxicity and Carcinogenicity held 10–17 January 1993 in Madonna di Campiglio, Italy.

This work was supported in part by the Fukuoka Cancer Society, Ishibashi Research Foundation, Uehara Memorial Foundation, and Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan. The authors thank Mie Kitajima for her excellent secretarial assistance.

Address correspondence to Masayasu Sugiyama, Department of Medical Biochemistry, Kurume University School of Medicine, 67 Asahi-machi, Kurume, 830 Japan. Telephone 81 942 31 7544. Fax 81 942 31 4377.

Materials and Methods

Hydrogen peroxide-resistant Chinese hamster ovary (CHO^R) cells were derived from the parental (CHO^P) cells by adding progressively higher concentrations (6.25 to 100 μM) of H_2O_2 in stepwise fashion to the culture medium (*12*). Logarithmically growing cells were treated with H_2O_2 for 1 hr or with Na_2CrO_4, $CdCl_2$, and $HgCl_2$ for 2 hr in salts-glucose medium [50 mM Hepes (pH 7.2) containing 10 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 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_2CrO_4, 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_2O_2 that reduced colony formation by 50% (ID_{50}) was 5-fold less in CHO^R cells than in CHO^P cells. CHO^R were also cross-resistant to $CdCl_2$ and $HgCl_2$, but not to Na_2CrO_4.

Table 2 shows the induction of DNA single-strand breaks produced by H_2O_2 or the metal compared in CHO^R and CHO^P cells. The formation of DNA breaks induced by H_2O_2 was almost completely inhibited in CHO^R cells. Although the cytotoxicity was not effected, the formation of DNA breakage produced by Na_2CrO_4 was significantly reduced by about 50% in CHO^R cells as compared to that in CHO^P cells. CHO^R were also cross-resistant to DNA breaks caused by $CdCl_2$ and $HgCl_2$.

It is unlikely that the observed metal-resistance in CHO^R 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

Table 1. Hydrogen peroxide and metal sensitivity of CHO^P and CHO^R cells.

Treatment	ID_{50} (μM)[a] CHO^P	CHO^R
H_2O_2	34	175
Na_2CrO_4	15.5	14.7
$CdCl_2$	6.1	1.6
$HgCl_2$	0.66	0.26

[a]ID_{50} values are the concentrations that inhibit colony formation by 50% after treatment with H_2O_2 for 1 hr, or with the metals for 2 hr.

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

Treatment[a]	μM	DNA single-strand breaks CHO^P	CHO^R
H_2O_2	25	0.663 ± 0.058[b]	0.051 ± 0.006[c]
Na_2CrO_4	400	0.505 ± 0.053	0.287 ± 0.068[c]
$CdCl_2$	300	0.491 ± 0.030	0.041 ± 0.019[c]
$HgCl_2$	2	0.510 ± 0.082	0.164 ± 0.096[c]

[a]Cells were treated with H_2O_2 for 1 hr or with the metals for 2 hr and then DNA breaks were measured as described (*13,14*). [b]Each value is the mean ± SD. [c]$p<0.01$ (Student's *t*-test).

chromium(VI) (*1–3*). In fact, relatively long-lived chromium(V) 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_2CrO_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 cross-resistant to the damage caused by $CdCl_2$ and $HgCl_2$. However, in the case of chromium(VI), CHO^R were found to be resistant only to DNA breaks, but not to the cytotoxicity caused by Na_2CrO_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^P 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 effected; 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.

REFERENCES

1. De Flora S, Wetterhahn KE. Mechanisms of chromium metabolism and genotoxicity. Life Chem Rep 7:169–244 (1989).
2. De Flora S, Bagnasco M, Serra D, Zanacchi P. Genotoxicity of chromium compounds: a review. Mutat Res 238:99–172 (1990).
3. Sugiyama M. Role of physiological antioxidants in chromium(VI)-induced cellular injury. Free Rad Biol Med 12:397–407 (1992).
4. Ochi T, Ishiguro T, Ohsawa M. Participation of active oxygen species in the induction of DNA single-strand scissions by cadmium chloride in cultured Chinese hamster cells. Mutat Res 122:169–175 (1983).
5. Cantoni O, Christie NT, Swann A, Drath DB, Costa M. Mechanism of $HgCl_2$ toxicity in cultured mammalian cells. Mol Pharmacol 26:360–368 (1984).
6. Snyder RD. Role of active oxygen species in metal-induced DNA strand breakage in human fibroblasts. Mutat Res 193:237–246 (1988).
7. Kawanishi S, Inoue S, Sano S. Mechanism of DNA cleavage-induced by sodium chromate(VI) in the presence of hydrogen peroxide. J Biol Chem 261:5952–5988 (1986).
8. Kortenkamp A, Ozolins Z, Beyersmann D, O'Brien P. Generation of PM2 DNA breaks in the course of reduction of chromium(VI) by glutathione. Mutat Res 216:19–26 (1989).
9. Kortenkamp A, Oetken G, Beyersmann D. The DNA cleavage induced by a chromium(V) complex and by chromate and glutathione is mediated by active oxygen species. Mutat Res 232:155–161 (1990).
10. Aiyar J, Berkovits HJ, Floyd RA, Wetterhahn KE. Reaction of chromium(VI) with hydrogen peroxide in the resonance of glutathione: reactive intermediates and resulting DNA damage. Chem Res Toxicol 3:595–603 (1990).
11. Shi X, Dalal NS. Evidence for a Fenton-type mechanism for the generation of •OH radicals in the reduction of Cr(VI) in cellular media. Arch Biochem Biophys 281:90–95 (1990).
12. Sugiyama M, Tsuzuki K, Haramaki N. DNA single strand breaks and cytotoxicity induced by sodium chromate(VI) in hydrogen peroxide-resistant cell lines. Mutat Res 299:95–102 (1993).
13. Sugiyama M, Costa M, Nakagawa T, Hidaka T, Ogura R. Stimulation of poly (adenosine diphosphoribose) synthesis by DNA lesions induced by sodium chromate in Chinese hamster V-79 cells. Cancer Res 44:1100–1104 (1988).
14. Sugiyama M, Tsuzuki K, Ogura R. Effect of ascorbic acid on DNA damage, cytotoxicity, glutathione reductase, and formation of paramagnetic chromium in Chinese hamster V-79 cells treated with sodium chromate(VI). J Biol Chem 266:3383–3386 (1991).
15. Sugiyama M, Ando A, Ogura R. Effect of vitamin E on survival, glutathione reductase and formation of chromium(V) in Chinese hamster V-79 cells treated with sodium chromate(VI). Carcinogenesis 10:737–741 (1989).