Role of Paramagnetic Chromium in Chromium(VI)-induced Damage in Cultured Mammalian Cells

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Chromium(VI) compounds are known to be potent toxic and carcinogenic agents. Because chromium(VI) is easily taken up by cells and is subsequently reduced to chromium(III), the formation of paramagnetic chromium such as chromium(V) and chromium(II) is believed to play a role in the adverse biological effects of chromium(VI) compounds. The present report, uses electron spin resonance (ESR) spectroscopy; the importance of the role of paramagnetic chromium in chromium(VI)-induced damage in intact cultured cells is discussed, based upon our studies with antioxidants including vitamin E (a-tocopherol), B2 (riboflavin), C (ascorbic acid), and so on. These studies appear to confirm the participation of paramagnetic Cr such as chromium(V) and Chromium(III) in chromium(VI)-induced cellular damage. — Environ Health Perspect 102(Suppl 3):31–33 (1994).

Key words: chromium(VI), paramagnetic chromium, DNA damage, antioxidants

Introduction

Chromium(VI) compounds are well-established human carcinogens based upon epidemiologic studies (1). They induce chromosomal aberrations, mutations, and transformation in cultured mammalian cells (2–4). Earlier studies have reported that chromium(VI) compounds produced a variety of DNA lesions such as DNA single-strand breaks, alkali-labile sites, and DNA-protein crosslinks, as well as selectively inhibiting the activity of enzymes such as glutathione reductase (2–4).

Chromium(VI) compounds have been shown to be more toxic and carcinogenic than chromium(III) compounds, because they readily enter cells through the sulfate transport system (4). However, after entering the cell, chromium(VI) is reduced to trivalent form, through chromium(V) and (IV) intermediates, by cellular reductants such as ascorbic acid, riboflavin, glutathione and flavoenzymes, including cytochrome P-450 reductase and glutathione reductase (2–4).

Thus, the levels of these biological reductants and paramagnetic chromium species in inside cells might be associated with the induction of chromium(VI)-induced damages. Therefore, electron spin resonance (ESR) studies have focused on the formation of paramagnetic chromium species such as chromium(V) and -(III) during reduction of chromium(VI) by biological reductants in vitro. The studies showed that this reduction process generates radical species such as active oxygen species, as well as glutathionyl radicals, with concomitant formation of chromium(V) species (4–12). In addition, previous studies reported that chromium(V) complex induced DNA breaks in vitro, and mutations in bacterial systems (7,9,13,14). Furthermore, recent in vitro studies have shown that biologically generated chromium(V) complexes react with hydrogen peroxide (H2O2), in a Fenton-type manner, to produce more hydroxyl radicals than a similar reaction with chromium(VI) (9,10,15). However, in intact cells, there have been very few studies that examined the role of paramagnetic chromium in the genotoxicity and toxicity caused by chromium(VI) compounds (16,17).

The present report discusses the relationship between paramagnetic chromium and chromium(VI)-induced damages in intact cultured cells using ESR spectrometry, based upon our study with the modification of chromium(V) and chromium(III) by reductants (18–26). The results indicate the important role of paramagnetic chromium in the genotoxicity and toxicity of chromium(VI) compounds in cells.

Formation of Chromium(V) and Chromium(III) in Intact Cells

Incubation of cultured Chinese hamster V-79 cells with Na2CrO4 resulted in the appearance of the ESR signals of both chromium(V) and Cr(III) complex, as measured by ESR spectroscopy at a temperature of 153° K (Figure 1). The signal of chromium(V) was observed with an anisotropy at g = 2.016 and g* = 1.989, and the line width of the maximum absorption peak was 12 to 13G (22,23,26). On the other hand, a rather broad signal with a g value of 2.02 to 2.03 and a line width of 700 to 800G was also observed concomitantly with that of the chromium(V) signals (21–23,26). The formation of both chromium(V) and -(III) complex in cells was found to increase proportionally to the concentration of chromate (50 to 500 μM) as well as to the time of exposure (30 min to 2 hr) to this metal (26). Since the line width of the chromium(III) hexaaqueous complex signal has been reported to be about 150G (17), it is possible that the increased line width of chromium(III) in the cells may be related to the formation of chromium(III) complex with cellular components, leading to reduced mobility of this metal (26).

As shown in Figure 1, when the relative concentration of paramagnetic chromium species in cells was estimated by analyzing the areas of the ESR signals, the concentrations of chromium(III) were about 30 times greater than those of chromium(V), indicating that most of the chromium inside...
the cells was in the form of chromium(III) (22,26). Following removal of extracellular
complex, the level of chromium(V) complex decreased quickly during the first
hour but more slowly during the next hour, whereas the level of chromium(III)
remained unchanged (26). Thus it appears that chromium(VI) may be
subsequently reduced through chromium(V) to (III) complex in intact
cells.

These studies showed that paramagnetic chromium species formed during
reduction could be clearly detected in intact cultured cells, as measured by ESR
spectroscopy.

Modification of the Levels of Paramagnetic Chromium and
Chromium(VI)-induced Damage in V-79 Cells

Among biological reductants, an increase of the content of vitamins such as α-toco-
opherol, riboflavin, and ascorbic acid by the pretreatment of V-79 cells with
tocopherol succinate, riboflavin, and ascorbic acid, respectively, resulted in alteration of
the levels of paramagnetic chromium species, as estimated by ESR spectroscopy (18,
20–23,27). As shown in Table 1, an increase of either α-tocopherol or ascorbic
acid in cells reduced the level of chromium(V) complex, whereas an increase of riboflavin enhanced the level of
this intermediate (20–23). With respect to chromium(III), elevated ascorbic acid
content was only found to increase chromium(III) content (20,22,23).

Under the same experimental condi-
tions, the induction of DNA damage,
enzyme inhibition and cytotoxicity by
Na2CrO4, as well as cellular uptake of this
metal were investigated, as summarized in
Table 1. DNA single strand breaks and/or
alkali-labile sites induced by chromate were
decreased by increasing the levels of α-
tocopherol or ascorbic acid, while increased
riboflavin content enhanced the levels of
both levels of DNA damage (18,19,21–23).
Similarly, the elevation of
α-tocopherol or ascorbic acid in cells
restored the glutathione reductase activity
suppressed by chromate, and an increase of riboflavin enhanced this enzyme inhibition
(Table 1) (20–23).

Recently, we also found that the cell-per-
meable metal chelator α-phenanthroline
(αP) suppressed the formation of
chromium(V) intermediates, as evaluated by
ESR spectroscopy at room temperature,
resulting in a decrease of chromate-induced
dNA breaks and/or alkali-labile sites, as
well as in a recovery of glutathione reduc-
tease inhibited by this metal in V-79 cells
(28). In addition, hydrogen peroxide-resis-
tant Chinese hamster ovary (CHO) cells
were found to have less total chromium(V)
and simultaneously fewer DNA strand
breaks than those in the parental cells (29).

In all the cases mentioned above, cellular
levels of chromium(V) were correlated with
the levels of chromate-induced DNA sin-
gle-strand breaks, alkali-labile sites, and
the enzyme inhibition.

We further investigated the effects of increased levels of α-tocopherol or
riboflavin on the induction of chromosom-
al aberrations and mutations at HGPRT
locus by chromium(VI) in V-79 cells (24,
25). The results showed that an increase of
α-tocopherol suppressed the clastogenic
and mutagenic action of chromate com-
 pounds (24), while the increased riboflavin
resulted in an enhancement of both actions
of this metal (25). These results suggest
that chromium(V) may be associated with
the clastogenic and mutagenic activity of
chromate.

On the other hand, levels of DNA-pro-
tein cross-links produced by Na2CrO4 were
unaffected by an increase of α-tocopherol
or riboflavin in V-79 cells, but the levels
were enhanced by an increase of ascorbic
acid (Table 1) (19,22,23). The uptake of
chromate was also unchanged by the eleva-
tion of α-tocopherol or riboflavin, but an
increase of ascorbic acid caused an acceler-
atation of metal uptake (Table 1) (20–22).
Since we showed that most of the
chromium inside the cells was in the form
of chromium(III), and that ascorbic acid is
capable of increasing the cellular level of
chromium(III), these results suggest that
the formation of chromium(III) in cells
may be related to the level of DNA-protein
cross-links and cellular uptake of this
metal.

At the present time, the role of cellular
chromium(III) in the clastogenicity, muta-
genicity, and carcinogenicity of chromo-
ate(VI) is not well established. Although an
increase of ascorbic acid in cells caused an
increase of chromium(III) and a decrease of
chromium(V) levels, further study with
ascorbic acid could lead to a better under-
standing of the role of chromium(III) in
chromate(VI)-induced carcinogenicity.

With respect to cytotoxicity, as estimated by colony-forming assays, an increase of
α-tocopherol in V-79 cells caused a marked
reduction of cytotoxicity induced by
Na2CrO4 the increased levels of riboflavin
resulted in a decrease in the cell mortality
caused by this metal, while the cytotoxicity
of chromate was enhanced by the elevation
of ascorbic acid (Table 1) (20–24). Thus,

Table 1. Levels of paramagnetic chromium and chromate-induced
Damage in cultured cells.

| Increase of cellular content | Cr(V) | Cr(III) | DNA breaks and/or alkali-labile sites | Inhibition of glutathione reductase | DNA-protein crosslinks | Metal uptake | Cytotoxicity |
|-----------------------------|-------|--------|-------------------------------------|--------------------------------|------------------------|-------------|-------------|
| α-tocopherol                | (+)   | (0)    | (-)                                 | (-)                            | (0)                    | (0)         | (-)         |
| Riboflavin                  | (+)   | (0)    | (+)                                 | (+)                            | (0)                    | (0)         | (-)         |
| Ascorbic acid               | (-)   | (+)    | (-)                                 | (-)                            | (+)                    | (+)         | (+)         |

*The phenomena shown are increased (+), decreased (-), or unaltered (0) by increased levels of vitamins in V-79 cells.
PARAMAGNETIC CHROMIUM IN INTACT CELLS

Cultured mammalian cells was investigated utilizing ESR spectrometry. The results demonstrate that the formation of chromium(V) and - (III) can be clearly detected in cultured cells by ESR spectrometry and that the modification of cellular levels of these paramagnetic species by reductants such as vitamins influences the induction of the biological effects of chromium(VI) compounds. Based upon these results, intracellular chromium(V) appears to be the critical form that is responsible for DNA single-strand breaks, alkali-labile sites, chromosomal aberrations, and mutation, as well as for the inhibition of glutathione reductase. Intracellular chromium(III) may be the critical form responsible for the DNA-protein crosslinks induced by chromium(VI) compounds. In contrast, the formation of chromium(III) and chromium(V) may not be directly related to the metal-induced cytotoxicity. Since ESR studies showed that chromium(III) may be subsequently reduced to chromium(III) in cultured cells, it is necessary to elucidate the role of the ultimate cellular form, chromium(III), in the mutation and carcinogenicity of chromium(VI) compounds. However, a correlation between the level of chromium(V) intermediate and chromium(VI)-induced mutation was detected in cultured cells. Therefore, paramagnetic chromium, in particular chromium(V), may play an important role in the induction of carcinogenicity by chromium(VI) compounds.

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