0026-895X/86/020173-06$02.00/0
Copyright © by The American Society for Pharmacology and Experimental Therapeutics.
All rights of reproduction in any form reserved.
MOLECULAR PHARMACOLOGY 29:173–178

MOL PHARM

Differences in the Effects of Hg(II) on DNA Repair Induced in Chinese Hamster Ovary Cells by Ultraviolet or X-Rays

NELWYN T. CHRISTIE,[1] ORAZIO CANTONI, MASAYASU SUGIYAMA, FLAMINIO CATTABENI, and MAX COSTA[1]

Department of Experimental Pediatrics, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030 (N.T.C.), Instituto di Farmacologia e Farmacognosia, Universita degli Studi di Urbino, 61029 Urbino, Italy (O.C., F.C.), and Department of Pharmacology, University of Texas Medical School at Houston, Houston, Texas 77025 (M.S., M.C.)

Received August 14, 1985; Accepted November 14, 1985

SUMMARY

The effect of relatively nontoxic levels of $HgCl_2$ on semiconservative DNA synthesis and on DNA repair induced following treatment of intact cells with X-ray or ultraviolet (UV) light has been studied in cultured Chinese hamster ovary cells. In the presence of 1 μM $HgCl_2$ the repair of DNA strand breaks induced by 450 rads of X-rays was reduced by 37%. If a treatment of 2.5 μM $HgCl_2$ was given to cells for only 15 min prior to a 450-rad irradiation, the rate of repair was reduced even further with only 25% of the breaks being repaired in the first hour following irradiation. When comparable treatments of $HgCl_2$ were given to Chinese hamster ovary cells in conjunction with UV irradiation there was no significant effect on either the number of initial strand scission events or the return to high molecular weight DNA following completion of repair. Only after exposure of cells to toxic levels of Hg(II) (higher concentrations or longer treatments) was there measurable inhibition of UV-induced repair as evidenced by a reduced rate of ligation of DNA to a high molecular weight form. Inhibition of the endonuclease step of UV repair was not observed since Hg(II)-treated cells exhibited the same level of strand scisson immediately following UV as cells not treated with Hg(II). The observed differences in the effects of Hg(II) on two pathways for DNA repair indicate that the potential for synergistic action between Hg(II) and other DNA damaging agents will be determined in part by the repair pathways induced by each agent. Additionally, it was found that inhibition of semiconservative synthesis also occurs at low concentrations of $HgCl_2$ similar to those affecting X-ray-induced repair. The presence of Hg-DNA adducts in the DNA at these concentrations may cause a reduction in normal replication to facilitate DNA repair.

Numerous metal ions have been clearly established as carcinogens either in animal studies, e.g., cadmium and lead, or by human epidemiological studies, e.g., chromium and nickel (1). In contrast to these studies in which the metal compound is thought to act as an initiator in the process of cellular transformation and carcinogenesis, other studies suggest a co-carcinogenic role for metal compounds. Examination of the incidence of lung cancer in metal refinery workers has suggested that nickel may act synergistically with polycyclic aromatic hydrocarbons in the production of respiratory carcinogenesis (2–4). Synergistic activity of nickel as well as cadmium and chromium has been demonstrated when given in combination with benz(*a*)pyrene yielding an enhanced frequency of cellular transformation. In an *in vitro* assay using primary Syrian hamster embryo cells, the synergistic response occurred at concentrations of metal compounds producing little or no toxicity or cell transformation when given alone (5, 6).

An effect on the rate or fidelity of DNA polymerization is a plausible target for metal action, and a correlation has been shown between carcinogenic metals and their ability to alter the fidelity of various DNA and RNA polymerases in test systems using purified enzymes (7, 8). The observed effect of metals on polymerase action suggests that metal ions may enhance the potency of carcinogens by alteration of cellular processes directly or indirectly involved in DNA repair. An alteration in the rate of repair of DNA damage produced by carcinogens may be sufficient to produce an enhanced rate of transformation. If metals prolong the time that strand breaks remain in the DNA following X-ray damage or endonuclease cleavage in the excision repair of UV-induced DNA damage, DNA recombinations may occur and lead to chromosomal rearrangements which have been associated with neoplasia.

In contrast to metals of known carcinogenic potential such as nickel and chromium, there is little evidence to suggest a

This work was supported by Grant CA 29581 from the National Cancer Institute, by Research Contract DE-A505-81ER60016 from the United States Department of Energy and from an allocation of the University Cancer Foundation, and by a grant from Regione Marche.

[1] Present address: Department of Environmental Medicine, NYU Medical Center, 550 First Avenue, New York, NY 10016.

ABBREVIATIONS: CHO cells, Chinese hamster ovary cells; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; SSF, strand scission factor; RF, rejoining factor; araC, arabinosylcytosine; HU, hydroxyurea.

MOLECULAR PHARMACOLOGY
aspet
Downloaded from molpharm.aspetjournals.org at Universidade do Estado do Rio de Janeiro on December 5, 2012

mutagenic or carcinogenic role for inorganic mercury (1, 9, 10). The absence of these genotoxic responses appears inconsistent with observations of DNA damage following exposure to inorganic mercury. In studies using cultured CHO cells we have previously shown that inorganic mercury produces single strand breaks and DNA/DNA crosslinks at concentrations that also cause cell lethality (11). In contrast to the DNA damage observed at relatively high concentrations, nontoxic levels of $HgCl_2$ did not produce measurable levels of strand scission but have been shown to reduce the rate of repair of single strand breaks induced by X-rays (12). The observed effect of Hg(II) on one pathway for DNA repair has prompted further study to examine the effect of $HgCl_2$ on the repair of UV-induced lesions as well as on semiconservative synthesis of DNA.

The repair of X-ray-induced damage is generally considered to occur by a different pathway than repair of UV-induced pyrimidine dimers (13, 14). Three mammalian DNA polymerases have been described, and polymerase α has been implicated as the principal polymerase for normal replication (15) as well as being the polymerase involved in the repair of DNA lesions induced by UV and certain alkylating agents (16, 17). Polymerase β is considered responsible for repair of X-ray-induced strand breaks as well as being involved in the repair of UV-induced lesions (16–18). By analyzing the effects of Hg(II) on DNA replication and repair, we have found that these parameters are in general a more sensitive indicator of Hg(II) toxicity than are other responses associated with metal toxicity such as DNA damage and depletion of reduced glutathione levels.

Materials and Methods

Cell culture techniques. The AA8 CHO cell line used in this study has been characterized as competent for excision repair induced by UV light (19). Cell maintenance and growth experiments were performed using α-minimal essential medium or McCoy's 5a medium containing 1% antibiotic-antimycotic mixture (Gibco, Inc., Grand Island, NY) and 10% fetal bovine serum (Gibco) that had been heat-inactivated at 56° for 30 min. Cultures were grown as monolayers in an atmosphere of 95% air and 5% CO_2. In some cases cells were treated with $HgCl_2$ in a salts/glucose medium [Hepes buffer (50 mM at pH 7.2 containing 100 mM NaCl), KCl (5 mM), $CaCl_2$ (2 mM), and glucose (5 mM)] to achieve more consistency in the extent of Hg(II) uptake than was possible in the presence of serum or amino acid components of medium (20).

Alkaline elution. The alkaline elution technique for analysis of DNA strand breaks was performed with minor modifications as previously described (11, 21). Following treatment, cells were removed with trypsin, and an aliquot containing 8.5×10^5 cells was diluted to 10 ml with ice-cold Puck's saline A (4 mM $NaHCO_3$, 5 mM glucose, 5 mM KCl, and 140 mM NaCl, pH 7.2) and deposited onto 25-mm polycarbonate filters (2 μm pore size). Filters were rinsed with 10 ml of saline A, and the cells were lysed directly on the filters by passage of 5 ml of 2% sodium dodecyl sulfate containing 0.025 M ethylenediaminetetraacetate at pH 10.1. The DNA was eluted from the filter by slowly pumping (0.035 ml min^{-1}) a solution (25 ml) containing 0.02 M ethylenediaminetetraacetate (free acid) plus 2% (final concentration) tetrapropylammonium hydroxide, pH 12.1. Fractions were collected at 90-min intervals. Following the completion of the elution procedure, the filters were digested for 1 hr at 60° in 1 N HCl and counted in 10 ml of Scintiverse II. The variability in the determination of the DNA fraction eluted was typically ≤ 0.06.

To quantitate the extent of DNA single strand breaks, the SSF was calculated from the alkaline elution patterns utilizing the following relationship: SSF = log f_A/f_B, where f_A is the amount of DNA retained in the sixth fraction of the untreated sample and f_B is DNA retained in the sixth fraction of the treated samples. The rejoining factor (RF) was calculated as the log (f_t/f_0) where f_0 is the DNA retained in fraction 6 at zero time after the pulse for untreated cells, and f_t is the DNA retained in fraction 6 at a given time after the pulse for cells that are either treated or untreated during the time interval for rejoining. The values for SSF and RF cannot be compared directly since two different processes are being monitored: production of strand breaks in template DNA and interruption of synthesis of newly formed strands. The choice of the sixth fraction collected after 7.5 hr of elution is somewhat arbitrary but does reduce the influence of the dead volume effect in the first fraction and avoids the poor statistics at the very low proportions of DNA retained in later fractions.

Assay of DNA synthesis. Normal replicative DNA synthesis was monitored by the incorporation of [^{3}H]thymidine into DNA. After exposure of cells to radiolabel, cells were trypsinized, washed with saline A, and treated with 10% trichloroacetic acid to precipitate the DNA. The precipitates were collected on 0.45-μm filters (Millipore Corp., Bedford, MA), and the radioactivity present on each filter was determined.

Results

The cytotoxic response of cultured CHO cells to $HgCl_2$ has been shown to involve a sequential pathway of lowered levels of reduced glutathione, increased intracellular levels of oxygen radicals, and the production of DNA strand breaks and DNA/DNA crosslinks (11, 22–23). At concentrations lower than those required to produce any of the above effects, Hg(II) ions have been shown to inhibit repair of strand breaks induced by X-rays (12, 24). This cellular response has been further characterized by examining the repair of strand breaks following 450 rads of X-rays in the presence of 1.0 μM and 2.5 μM $HgCl_2$ in a salts/glucose medium (Fig. 1). In the absence of $HgCl_2$, the strand breaks have been essentially removed (~96% restoration) in 1 hr whereas restoration of high molecular weight DNA is 63% at 1 μM and only 28% in 2.5 μM $HgCl_2$. During the 2.5 μM $HgCl_2$ treatment, strand break repair seemed to reach a plateau after 30 min of exposure. DNA strand scission was minimal at 2.5 μM $HgCl_2$ (SSF $\leq$ 0.03) and thus was not expected to be a contributing factor in the level of breaks retained during the time allocated for repair (22).[2] The repair of strand breaks induced by X-rays continued in the presence of 1 μM $HgCl_2$ but the rate of repair was continually reduced with time. The effect of Hg(II) on X-ray-induced repair was examined further by pretreating monolayers of CHO cells with 2.5 or 5 μM $HgCl_2$ 15 min before irradiation and then permitting repair to take place in complete medium not containing the metal salt (Fig. 2). This brief treatment at 2.5 μM $HgCl_2$ produced a slight increase in the initial levels of X-ray-induced strand scission, presumably because Hg(II) entered cells quickly and inhibited DNA polymerization, whereas in the absence of Hg(II) some repair of X-ray-induced strand breaks occurred prior to cell lysis. Strand break repair in the presence of 2.5 μM $HgCl_2$ was significantly slower than in normal medium. The elevated initial level of strand scission in the cells treated with 5 μM $HgCl_2$ treatment over the level in cells treated with X-rays alone, as well as the continued increase in breaks with time, suggested both additivity of strand scission events for the two agents and inhibition of the repair of breaks induced by both agents. This was consistent with previous findings in which strand scission increased for 1 hr after removal of $HgCl_2$

[2] O. Cantoni, N. T. Christie, S. H. Robison, and M. Costa, unpublished observations.

MOL PHARM
MOLECULAR PHARMACOLOGY
aspet
Downloaded from molpharm.aspetjournals.org at Universidade do Estado do Rio de Janeiro on December 5, 2012

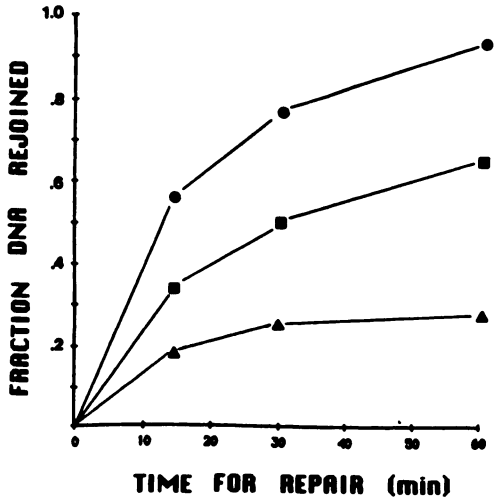

Fig. 1. The effect of $HgCl_2$ on the repair of X-ray-induced strand breaks. CHO cells were irradiated with 450 rads and allowed varying periods of time for repair at room temperature in salts/glucose medium containing no $HgCl_2$ (●——●), 1 μM $HgCl_2$ (■——■), or 2.5 μM $HgCl_2$ (▲——▲). DNA strand breaks were detected by alkaline elution. From SSF values calculated as described in Materials and Methods, the DNA fraction rejoined was calculated as the ratio of the change in strand scission with time to the total strand scission produced by 450 rads at t_0, e.g., $[(SSF)_0 - (SSF)_t]/(SSF)_0$. Total initial strand scission is represented by $(SSF)_0$ and strand scission at any later time for any treatment is given by $(SSF)_t$. The value of $(SSF)_0$ was 0.65 ± 0.03. The data of Fig. 1 are averages of two determinations but comparable results were found in three other experiments.

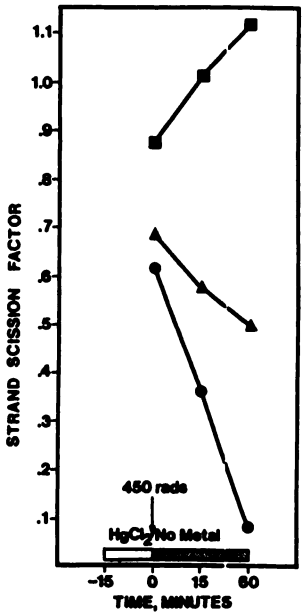

Fig. 2. The effect of preincubation with $HgCl_2$ on the repair of X-ray-induced strand breaks. CHO cells were preincubated at room temperature for 15 min in salts/glucose medium containing no $HgCl_2$ (●——●), 2.5 μM $HgCl_2$ (▲——▲), or 5 μM $HgCl_2$ (■——■). Alkaline elution was performed and an SSF was calculated as described in Materials and Methods. The *bars* at the *bottom* of the figure indicate the time for metal treatment when present.

if the initial treatment was high enough to produce measurable levels of oxygen radicals (22, 23).

The effect of Hg(II) on semiconservative synthesis was also examined. Alteration in the normal replicative function of DNA polymerase α may result from DNA damage, from direct effects of $HgCl_2$ on the enzyme, or from indirect effects by interference with critical ions needed for polymerization, e.g., Mg(II). Assays for replication were performed for time periods of 15 min and 1 hr of $HgCl_2$ treatment followed by 1 hr to allow incorporation of ^{3}H-thymidine as a quantitative estimate of synthetic activity (Fig. 3). At 1 μM $HgCl_2$ for 1 hr, the level of synthesis was 15% of untreated cultures which represented a level of inhibition that was considerably greater than that observed for Hg(II) inhibition of X-ray-induced repair (67%, Fig. 1). A 15-min treatment of 2.5 μM $HgCl_2$ reduced semiconservative synthesis to 11% of that for untreated cells. A similar Hg(II) pretreatment reduced the amount of X-ray repair occurring in 1 hr to ~30% compared to ~85% for cells receiving X-rays without Hg(II) pretreatment, as measured by a ratio of the SSF at the beginning and the end of the repair period (Fig. 2). Thus, at concentrations of 1.0 and 2.5 μM $HgCl_2$ normal replication appeared more sensitive than repair synthesis.

An additional measure of Hg(II) effects on semiconservative synthesis was also utilized and involved a pulse of ^{3}H-thymidine 20 min before the addition of varying concentrations of $HgCl_2$ in a salts/glucose medium (Fig. 4). During a brief labeling period, Okasaki fragments of low molecular weight will be synthesized and gradually converted to high molecular weight DNA by progressive synthesis and ligation (25). The strand breaks observed by this labeling method are only in newly synthesized DNA rather than in template DNA as monitored in Figs. 1 and 2. In Fig. 4A it can be seen that treatment with 0.5 μM $HgCl_2$ reduced the rate of this process to 88% compared to untreated cells, whereas 2.5 μM $HgCl_2$ reduced it to 27%. When a concentration of 1 μM $HgCl_2$ was used and the relative increase in size was monitored at regular time intervals, the increase in DNA size was reduced with time of exposure to Hg(II) at a steady rate for the first 15 min after which no further increase in the inhibitory response was observed (Fig.

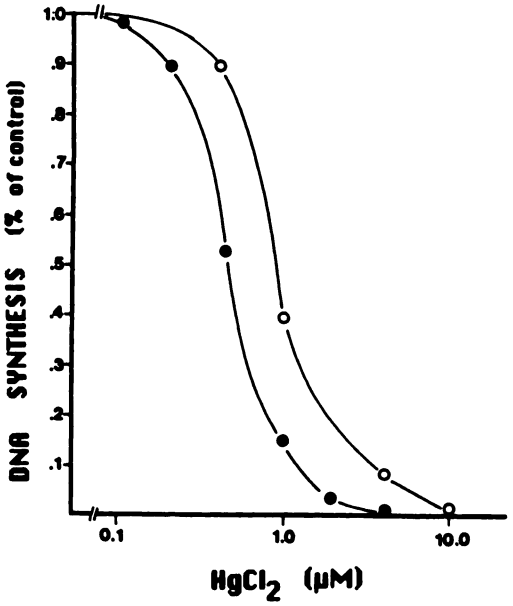

Fig. 3. The effect of $HgCl_2$ on normal replicative DNA synthesis. Monolayers of CHO cells were treated with $HgCl_2$ in salts/glucose medium for 15 min (○) or 1 hr (●) before addition of McCoy's medium containing ^{3}H-thymidine (1 μCi/ml) for 1 hr. The total amount of DNA synthesis was determined as described in Materials and Methods.

MOL PHARM
MOLECULAR PHARMACOLOGY
aspet
Downloaded from molpharm.aspetjournals.org at Universidade do Estado do Rio de Janeiro on December 5, 2012

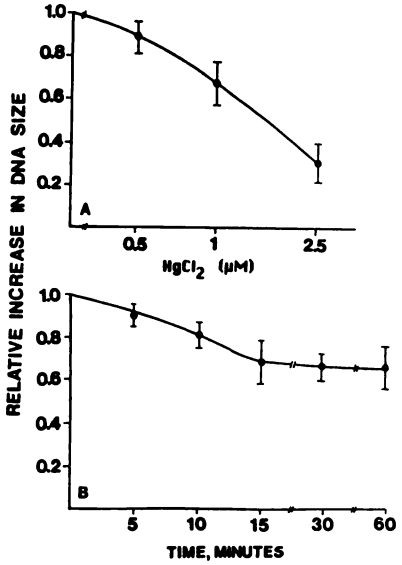

Fig. 4. The effect of $HgCl_2$ on the rate of joining of newly synthesized DNA fragments during semiconservative synthesis. CHO cells were given a 20-min pulse of ^{3}H-thymidine (20 μCi/ml) (A) before addition of $HgCl_2$ at varying concentrations for 1 hr or (B) before addition of 1 μM $HgCl_2$ for varying periods of time. In each series the $HgCl_2$ was administered to cells in a salts/glucose medium. Some cell samples received the 20-min pulse without an addition of $HgCl_2$ to determine the maximum extent of rejoining for all time periods examined. The relative increase in DNA size was the ratio of the RF values for treated cells to that of untreated cells. Calculation of RF is given in Materials and Methods. The average RF for 1 hr for untreated cells is 0.51 ± 0.04.

4B). This implies saturation of some site of action. While this site may be the polymerase, it may also be initiation sites in DNA since the formation of Hg-DNA adducts occurred at low doses (4).

The extreme sensitivity to Hg(II) of semiconservative synthesis and DNA repair of strand breaks after X-irradiation prompted our investigation of Hg(II) effects on the repair of pyrimidine dimers produced by UV light. The same concentrations reducing X-ray repair (1, 2.5, and 5 μM $HgCl_2$) were used to test for possible inhibition of UV-induced repair. Fig. 5 shows the effect of 5 μM $HgCl_2$ in a salts/glucose medium added to monolayers of CHO cells 15 min before irradiation. After UV irradiation a 30-min period in salts/glucose was given during which strand scission occurred near pyrimidine dimers by a specific endonuclease as a first step in the removal of dimers. The maximum amount of observable strand scission was determined by the addition of 10 μM araC and 2 mM HU during the 15 min before and the 30 min after irradiation. The SSF for this maximum estimate of breaks was 0.84, whereas the SSF for cells receiving only UV light was 0.48, indicating that excision of some dimers had occurred during the repair period and was followed by polymerization and ligation. The addition of 5 μM $HgCl_2$ 15 min before UV irradiation had essentially no effect on the restoration of DNA to a higher molecular weight form. The lower concentrations also had no effect. Thus, these Hg(II) treatments did not appear to inhibit the polymerase or ligase steps to a significant degree. Since the Hg(II)-treated cells exhibited a level of strand scission that was similar to that of the treated cells, inhibition of the endonuclease step by Hg(II) was not apparent.

It is known that in the first 30 min–1 hr after UV irradiation there is a rapid generation of breaks; however, complete removal of all damaged regions of DNA and subsequent resynthesis occurs over many hours (26). We therefore examined a longer period for repair to determine whether inhibition could be measured. The effect of $HgCl_2$ on UV repair was determined for 1- and 3-hr time intervals in medium containing serum. Higher levels of $HgCl_2$ were utilized in complete medium so that comparable intracellular levels would be reached in the presence of serum (20). In Table 1 are the average SSFs obtained from several of these experiments and for several concentrations of $HgCl_2$ for the two time periods. Even after these longer periods for repair in the presence of Hg(II) there was no significant inhibition of UV repair. When a relatively high concentration of $HgCl_2$ was used, 150 μM for 1 hr, there was a significant increase in the SSF, but this increase can be attributed to direct breakage of DNA by Hg(II) (22, 23). The toxicity of this latter treatment was sufficient to inhibit growth of CHO cells by 50%, whereas 25 μM reduced growth by approximately 10% (11).

Discussion

A major manifestation of Hg(II) toxicity in cultured CHO cells was the production of DNA strand breaks after periods of exposure as short as 15 min (23). The cellular uptake of Hg(II) ions was quite rapid, possibly due to the formation of uncharged and negatively charged ion complexes with chloride that may facilitate passage of the metal through the plasma membrane. High intracellular levels of Hg(II) led to a reduction of glutathione levels and an increase in oxygen radicals, followed by production of DNA strand scission (11, 22, 23). The major burst of oxygen radicals occurred between 5 and 25 μM for a 30-min exposure in a balanced salts solution and coincided with observations of DNA strand scission (23). The cellular response for treatment at concentrations of $HgCl_2$ below those required to produce a significant elevation of oxygen radicals was more difficult to interpret. DNA repair in cultured CHO cells was induced at very low concentrations of $HgCl_2$, but the total repair activity was quite small relative to other metals such as

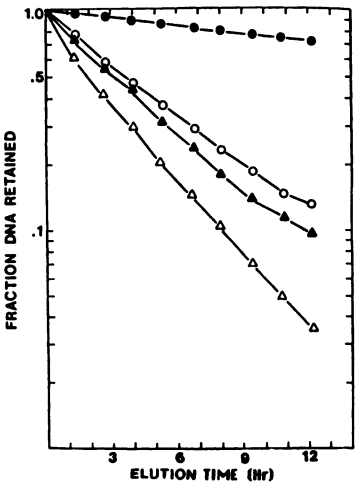

Fig. 5. The effect of Hg(II) on the rejoining of strand breaks. Several different treatments in salts/glucose were administered to CHO monolayers 15 min before UV irradiation of 5 J/M^2: ●, no treatment; ○, UV only; ▲, 5 μM $HgCl_2$ and UV; △, araC, HU, and UV, in which araC and HU were given 15 min before and after the irradiation. After irradiation each culture sample was allowed 30 min in salts/glucose without the above additives.

MOL PHARM
MOLECULAR PHARMACOLOGY
aspet
Downloaded from molpharm.aspetjournals.org at Universidade do Estado do Rio de Janeiro on December 5, 2012

TABLE 1
The effect of $HgCl_2$ on the repair of UV-induced pyrimidine dimers

Treatment	SSF[a]	
	Repair time after UV[b]	
	1 hr	3 hr
UV	0.5	0.39
UV + 15 μM Hg	0.58	0.36
UV + 25 μM Hg	0.46	0.33
UV + 50 μM Hg	0.57	0.46
UV + 150 μM Hg	0.7	

[a] The strand scission factor was calculated as indicated in Materials and Methods. Each value is an average of two to four determinations and the variation range is from 0.01 to 0.06.

[b] After UV radiation of CHO cell monolayers at a fluence of 5 J/m², α-MEM medium with or without $HgCl_2$ was applied for the times indicated before harvesting cells for alkaline elution.

$CaCrO_4$ (27). There was an apparent contradiction between this result and the finding that repair of X-ray-induced damage was inhibited at concentrations of Hg(II) that induced repair synthesis as measured by the CsCl density shift method. The repair synthesis observed following Hg(II) treatment of CHO cells indicated that Hg(II)-induced DNA damage was capable of stimulating DNA repair but only to a limited extent. This may indicate either that Hg(II) ions have an inhibitory effect on DNA repair or that the amount of DNA synthesis, i.e., the number of nucleotides, required to repair Hg-induced lesions is very small as it is for repair of strand breaks induced by X-rays. The inhibitory effect of Hg(II) on DNA repair at low concentrations appeared to be a reduction in the rate of repair but was only a temporary block of repair since most cells recovered from the Hg(II) treatments that produced this response.

Single strand breaks following Hg(II) treatment alone are believed to be caused by the production of oxygen radicals only after the level of glutathione has been reduced (22, 23). A more plausible lesion leading to stimulation of DNA repair may be the Hg-DNA adduct. Prior measurements of Hg-DNA complexes formed in cultured CHO cells showed significant levels of these complexes after 15 min of treatment with 1 μM $HgCl_2$ in a salts/glucose medium (23). At this concentration, no strand breaks could be detected by the alkaline elution method. The inhibition of repair of one type of lesion by the activation of a DNA repair system with a different pathway has been reported; the inactivation of nucleotide excision repair has been observed in CHO cells by exposure to an alkylating agent (28). In addition, several investigations have indicated that excision repair may inhibit semiconservative synthesis (29); thus, it is possible that repair of Hg-DNA adducts might have a similar effect.

Thus, there are at least two feasible mechanisms by which Hg(II) could inhibit DNA repair, either by interference between two DNA repair systems or by a more direct effect on repair enzymes. These mechanisms suggest two important sites of interaction by which Hg ions at low nontoxic concentrations could reduce the rate of DNA repair: the DNA molecule itself or the DNA repair enzymes. There is evidence for interaction with DNA at these low concentrations and Hg-DNA adducts have been demonstrated at concentrations causing inhibition of DNA strand break repair following X-rays (23). The failure of Hg(II) to inhibit repair of UV-induced DNA damage as effectively as araC and HU suggests that Hg(II) at low concentrations probably does not have a direct effect on DNA synthesis, at least that which is involved in UV repair. Since both α and β DNA polymerases are postulated to act during UV-induced repair of DNA (30–32), a direct effect of Hg(II) on both of these enzymes seems improbable. Although it is possible that a DNA polymerase other than α or β is involved in UV-induced repair, another possibility is equally feasible to explain the dramatic effect of nontoxic levels of Hg(II) on both X-ray-induced DNA repair and semiconservative synthesis in the absence of a similar inhibition of UV-induced repair. The extreme Hg(II) sensitivity of semiconservative DNA synthesis which is performed by DNA polymerase α may be related to the presence of Hg-DNA adducts since the presence of any lesion in DNA may create a temporary pause in normal replication to allow mobilization of DNA repair systems. The Hg(II) sensitivity of X-ray-induced repair may also be due to the presence of Hg-DNA adducts, especially if these two types of damage are repaired by the same pathway. The lack of Hg(II) sensitivity of UV-induced DNA repair under the conditions tested is consistent with the current idea that the repair pathways for UV and X-rays are functionally distinct (29, 30). Complete repair of X-ray-induced strand breaks can be extremely rapid (<10 min) and involves only a few nucleotides (<10), whereas complete repair of UV-induced thymine dimers can be very slow (48 hr or longer) and may involve nuclease excision and resynthesis of DNA stretches of 100 or more nucleotides (28, 29). Although Hg(II) ions inhibited UV-induced repair when given at higher concentrations or for longer times, these conditions produce toxicity and thus are not relevant as a comparison to the inhibition of X-ray-induced repair at nontoxic levels. It is also possible that Hg(II) may be more effective at inhibition of X-ray-induced repair because of a mechanism unrelated to DNA repair; X-rays cause depletion of glutathione and may thereby sensitize the cell to the cytotoxic effects of Hg(II).

References

1. Heck, J. D., and M. Costa. *In vitro* assessment of toxicity of metal compounds. I. Mammalian cell transformation. *Biol. Trace Element Res.* **4**:71–82 (1982).
2. Doll, R., L. G. Morgan, and F. E. Speizer. Cancers of the lung and nasal sinuses in nickel workers. *Br. J. Cancer* **24**:623–632 (1970).
3. Dixon J. R., D. B. Lowe, D. E. Richards, L. J. Cralley, and H. E. Stokinger. The role of trace metals in chemical carcinogenesis: asbestos cancers. *Cancer Res.* **30**:1068–1074 (1970).
4. Sunderman, F. W. Inhibition of induction of benz(*a*)pyrene hydroxylase by nickel carbonyl. *Cancer Res.* **27**:950–955 (1967).
5. Rivedal, E., and T. Sanner. Synergistic effects on morphological transformation of hamster embryo cells by nickel sulfate and benz(*a*)pyrene. *Cancer Lett.* **8**:203–208 (1980).
6. Rivedal, E., and T. Sanner. Metal salts as promoters of *in vitro* morphological transformation of hamster embryo cells initiated by B(a)P. *Cancer Res.* **41**:2950–2953 (1981).
7. Sirover, M. A., and L. A. Loeb. Metal-induced infidelity during DNA synthesis. *Proc. Natl. Acad. Sci. USA* **73**:2331–2335 (1976).
8. Mildvan, A. S., and L. A. Loeb. The role of metal ions in the mechanisms of DNA and RNA polymerases. *Crit. Rev. Biochem.* **61**:219–244 (1979).
9. Christie, N. T., and M. Costa. *In vitro* assessment of the toxicity of metal compounds. III. Effects of metals on DNA structure and function in intact cells. *Biol. Trace Element Res.* **5**:55–71 (1983).
10. Leonard, A., P. Jacquet, and R. R. Lauwery. Mutagenicity and tertogenesis of mercury compounds. *Mutat. Res.* **114**:1–18 (1983).
11. Christie, N. T., O. Cantoni, R. M. Evans, R. E. Meyn, and M. Costa. Use of mammalian DNA repair-deficient mutants to assess the effects of toxic metal compounds on DNA. *Biochem. Pharmacol.* **33**:1662–1670 (1984).
12. Cantoni, O., R. M. Evans, and M. Costa. Similarity in the acute cytotoxic response of mammalian cells to Hg(II) and x-rays; DNA damage and glutathione depletion. *Biochem. Biophys. Res. Commun.* **108**:614–619 (1982).
13. Hanawalt, P. C., P. K. Cooper, A. K. Ganesan, R. S. Lloyd, C. A. Smith, and M. E. Zolan. Repair responses to DNA damage: enzymatic pathways in *E. coli* and human cells. *J. Cell. Biochem.* **18**:271–283 (1982).
14. Setlow, R. B. DNA repair pathways, in *DNA Repair and Mutagenesis in Eukaryotes* (W. M. Generoso, M. D. Shelby, and F. J. de Serres, eds.). Plenum Press, New York, 45–56 (1980).

MOL PHARM
MOLECULAR PHARMACOLOGY
aspet
Downloaded from molpharm.aspetjournals.org at Universidade do Estado do Rio de Janeiro on December 5, 2012

15. Bollum, F. J. Mammalian DNA polymerases. *Prog. Nucleic Acid Res. Mol. Biol.* **15**:109–144 (1975).
16. Miller, M. R., and D. N. Chinault. The roles of DNA polymerases α, β, and γ in DNA repair synthesis induced in hamster and human cells by different DNA damaging agents. *J. Biol. Chem.* **257**:10204–10209 (1982).
17. Mattern, M. R., R. F. Paone, and R. S. Day III. Eukaryotic DNA repair is blocked at different steps by inhibitors of DNA topoisomerases and of DNA polymerases α and β. *Biochim. Biophys. Acta* **697**:6–13 (1982).
18. Bermudez, E. The inhibition of DNA repair in primary rat hepatocyte cultures by aphidicolin: evidence for the involvement of alpha polymerase in the repair process. *Biochem. Biophys. Res. Commun.* **109**:275–281 (1982).
19. Thompson, L. H., K. A. Brookman, L. E. Dillehay, C. L. Mooney, and A. V. Carrano. Hypersensitivity to mutation and sister-chromatid exchange induction in CHO cell mutant detective in incising DNA containing UV-lesions. *Somatic Cell Genet.* **8**:759–773 (1982).
20. Abbracchio, M. P., R. M. Evans, J. D. Heck, O. Cantoni, and M. Costa. The regulation of ionic nickel uptake and cytotoxicity by specific amino acids and serum components. *Biol. Trace Element Res.* **4**:289–301 (1982).
21. Kohn, K. W., R. A. G. Ewig, L. C. Erickson, and L. A. Zwelling. Measurement of strand breaks and crosslinks by alkaline elution, in *DNA Repair: A Laboratory Manual of Research Procedures* (E. C. Friedberg and P. C. Hanawalt, eds.). Mercel Dekker, New York, 379–401 (1981).
22. Cantoni, O., N. T. Christie, S. H. Robison, and M. Costa. Characterization of DNA lesions produced by $HgCl_2$ in cell culture systems. *Chem. Biol. Interact.* **49**:209–224 (1984).
23. Cantoni, O., N. T. Christie, A. Swann, D. B. Drath, and M. Costa. Mechanisms of $HgCl_2$ cytotoxicity in cultured mammalian cells. *Mol. Pharmacol.* **26**:360–368 (1984).
24. Cantoni, O., and M. Costa. Correlation of DNA strand breaks and their repair with cell survival following acute exposure to mercury (II) and X-rays. *Mol. Pharmacol.* **24**:84–89 (1983).
25. Pardee, A. B., R. Dubrow, J. L. Hamlin, and R. F. Kletzien. Animal cell cycle. *Annu. Rev. Biochem.* **47**:715–750 (1978).
26. Park, S. D., K. H. Choi, S. W. Hong, and J. E. Cleaver. Inhibition of excision repair of ultraviolet damage in human cells by exposure to methylmethanesulfonate. *Mutat. Res.* **82**:365–371 (1981).
27. Robison, S. H., O. Cantoni, and M. Costa. Analysis of metal-induced DNA lesions and DNA-repar replication in mammalian cells. *Mutat. Res.* **131**:173–181 (1984).
28. Park, S. D., and J. E. Cleaver. Inactivation of nucleotide excision repair in Chinese hamster ovary cells by exposure to an alkylating agent. *Photochem. Photobiol.* **35**:419–421 (1982).
29. Cleaver, J. E. Sensitivity of excision repair in normal human, xeroderma pigmentosum variant and Cockayne's syndrome fibroblasts to inhibition by cytosine arabinoside. *J. Cell. Physiol.* **108**:163–173 (1981).
30. Preston, R. J. Radiation damage to DNA and its repair in the formation of chromosome aberrations, in *Radiation Induced Chromosome Damage in Man* (T. Ishihara and M. S. Sasaki, eds.). Alan R. Liss, Inc., New York, 111–125 (1983).
31. Waters, R. Aphidicolin: an inhibitor of DNA repair in human fibroblasts. *Carcinogenesis* **2**:795–797 (1981).
32. Snyder, R., and J. D. Regan. Aphidicolin inhibits repair of DNA in UV-irradiated human fibroblasts. *Biochem. Biophys. Res. Commun.* **99**:1088–1094 (1981).

Send reprint requests to: Dr. Nelwyn T. Christie, Department of Environmental Medicine, NYU Medical Center, 550 First Avenue, New York, NY 10016.

MOL PHARM
MOLECULAR PHARMACOLOGY
aspet
Downloaded from molpharm.aspetjournals.org at Universidade do Estado do Rio de Janeiro on December 5, 2012