Vol. 108, No. 2, 1982 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
September 30, 1982 Pages 614-619

SIMILARITY IN THE ACUTE CYTOTOXIC RESPONSE OF MAMMALIAN CELLS TO MERCURY (II) AND X-RAYS: DNA DAMAGE AND GLUTATHIONE DEPLETION

Orazio Cantoni, R. Mark Evans and Max Costa

Div. of Toxicology
Dept. of Pharmacology
The Univ. of Texas Medical School at Houston
P.O.Box 20708, Houston, Texas 77025

Received August 19, 1982

Pronounced strand breakage of DNA analyzed by alkaline elution techniques was produced in intact Chinese hamster ovary cells by 25 μM $HgCl_2$ within 1 hr or 100 μM $HgCl_2$ within 15 min. $HgCl_2$-induced strand breakage was directly proportional to concentration up to 100 μM and to time within 1 hr. Levels of reduced glutathione decreased following $HgCl_2$ in parallel with the induction of DNA strand breakage. Evidence is presented that this rapid and pronounced induction of DNA strand breaks and other cytotoxic responses following acute exposure to $HgCl_2$ resembles the cellular effects of X-rays.

Considerable evidence indicates that Hg^{2+} is a mutagen (1,2) and has potential carcinogenic activity since it enhances viral transformation of Syrian hamster embryo cells (3) and induces chromosomal aberrations (4). X-rays are known to induce transformation (5), mutagenesis (6) and have been unequivically established as carcinogenic in humans and experimental animals (7). X-rays are known to rapidly induce strand breaks in the DNA (8,9); in fact, X-ray treatment of cells is used to shear cellular DNA for detection of crosslinking agents by alkaline elution analysis (10,11). The ability of Hg^{2+} to induce damage to the DNA in intact cells has not been extensively studied. Both Hg^{2+} and X-rays are known to deplete cellular levels of reduced glutathione (GSH) (12), resulting in the loss of a major defense system which protects cellular lipids and other components against attack by oxygen radicals. It is thought that X-rays induce DNA damage by the generation of electrophilic reactive species resulting in the formation of $OH^{\cdot}$ and H_2O_2 (13,14). Ionic mercury (II) is known to interact with

Abbreviations used: SSF, Strand Scission Factor; GSH, Reduced Glutathione; CHO, Chinese Hamster Ovary

0006-291X/82/180614-06$01.00/0

DNA at nitrogen atoms, replacing hydrogen in the binding between the thymine base. This interaction results in an increased helix stability and an increased diameter of the double helix approximating the difference between the atomic radius of hydrogen and mercury (15). This interaction of Hg^{2+} with DNA could not resemble the effects of X-rays and probably has relevance only in the long term cytotoxic effects of low concentrations of Hg^{2+}. However, acutely toxic levels of Hg^{2+} may result in DNA damage due to enhanced production of reactive oxygen intermediates.

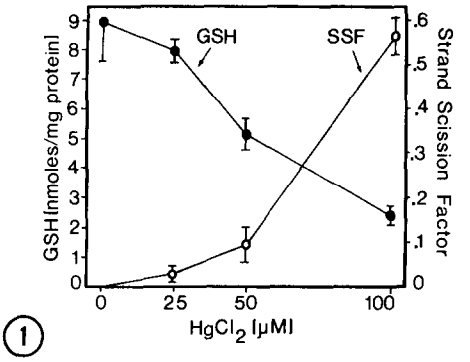
In the present study, we have examined the acute effects of Hg^{2+} on GSH levels and DNA strand breaks measured with alkaline elution, and have compared these and other parameters to the cellular perturbations caused by X-rays. Although further proof is required, considerable evidence suggest that the acute effects of $HgCl_2$ in intact cells mechanistically resembles the cellular effects of X-rays.

MATERIALS AND METHODS

Chinese hamster ovary cells were cultured in McCoy's 5A medium fortified with 10% fetal bovine serum (Gibco, Inc.) in monolayer culture in an atmosphere of 95% air and 5% CO_2. DNA strand breaks were determined in intact cells following treatment with $HgCl_2$ or X-rays by the alkaline elution procedure described by Kohn et al (10,11). DNA-protein crosslinking of $HgCl_2$ was assessed by proteinase K treatment of cell lysates as described by Kohn (10). Strand scission factor (SSF) was calculated from the alkaline elution patterns obtained by the following relationships: SSF = -log A/B. Where A = fraction of DNA retained in the 6th fraction of the untreated sample and B = DNA retained in the 6th fraction of treated sample. A total of nine, 90 min fractions were collected during the alkaline elution at a speed of 0.035 ml/min. GSH levels were estimated by a fluorometric assay using the dye O-phthaldehyde (16). Plating efficiency was determined following treatment of log-phase monolayer cultures with selected concentrations of $HgCl_2$ followed by trypsinization and replating of 100-10,000 cells in at least nine 60 mm plates for each concentration. The total number of surviving colonies in each plate was expressed as a function of the number of cells plated to assess plating efficiency. Double strand breakage of DNA was determined by neutral sucrose gradients as previously described (17). Metal concentration associated with cells was determined by X-ray fluoroscence spectroscopy previously described (18).

RESULTS

$HgCl_2$ treatment of cultured cells rapidly depleted the cellular levels of GSH and caused single strand breaks in the DNA (Fig. 1 and 2). Fig. 1 shows that both the depletion of GSH and the induction of strand breaks was dependent upon the concentration of $HgCl_2$ up to 100 μM following 1 hr of exposure. Plating efficiency values for 1 hr of exposure to 7.5, 10, 25, 50 and 75 μM $HgCl_2$ were



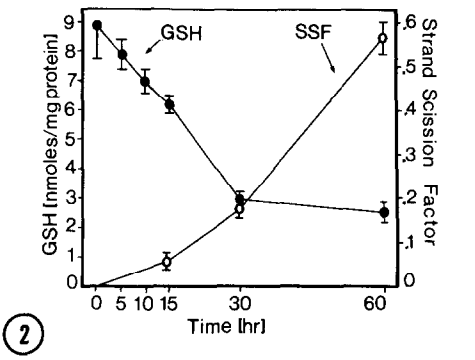


FIG. 1 Concentration dependent GSH depletion and DNA strand breaks induced by $HgCl_2$. Cultured CHO cells were exposed to the concentrations of $HgCl_2$ indicated in the figure for 1 hr. Cellular levels of GSH and the induction of DNA strand breaks were determined as described in the methods section.

FIG. 2 Temporal effects of $HgCl_2$ on depletion of GSH and induction of DNA strand breaks. CHO cells were exposed to 100 μM $HgCl_2$ for the time intervals indicated in the figure. Cellular GSH levels and induction of DNA strand breaks were determined as described in the methods section.

104%, 93.8%, 72.3%, 18.7% and 0 (% of untreated). These results indicated that $HgCl_2$ concentrations in excess of 25-50 μM for 1 hr were extremely cytotoxic to cells. This is not surprising in light of the extensive breakage of DNA caused by these concentrations (Fig. 1). GSH levels declined concomitantly with the induction of DNA strand breaks during the first hr of exposure to 100 μM $HgCl_2$ (Fig. 2). A more extensive time course study by alkaline elution analysis of DNA strand breaks induced by 50 μM Hg^{2+} revealed that most of the breaks occurred within the first hr of exposure (not shown). Neutral sucrose gradient analysis showed that the breakage of DNA induced by $HgCl_2$ (25-100 μM) was predominantly single stranded. $HgCl_2$ did not induce detectable DNA-protein crosslinks or interstrand crosslinking following analysis with alkaline elution in combination with proteinase K or X-ray treatment. The observation that combined treatment of cells with a variety of cytotoxic and DNA damaging concentrations of $HgCl_2$ and X-rays did not cause significantly more DNA strand breaks than each treatment alone, suggested a common mechanism of action and, possibly,

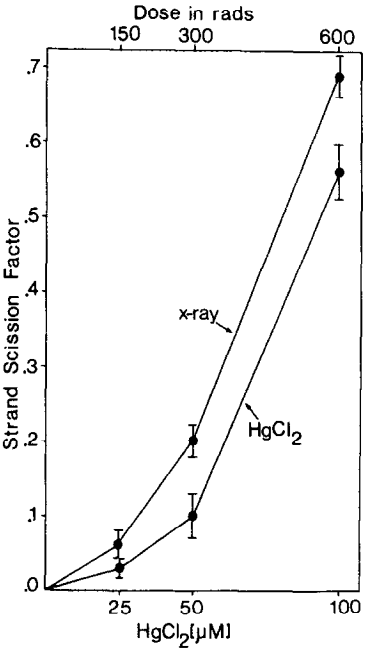


FIG. 3 Similarity in the induction of DNA strand breaks by $HgCl_2$ and X-rays. Alkaline elution was employed to determine the incidence of DNA strand breaks following 1 hr of exposure to the indicated concentrations of $HgCl_2$ or X-rays (see methods section).

a competition for cytotoxic target sites. Figure 3 shows the similarity in the rapid and pronounced induction of DNA strand breaks caused by $HgCl_2$ and X-rays. Addition of glutathione to the medium was able to inhibit $HgCl_2$ induced DNA strand breaks in intact cells (not shown) and cells with elevated levels of glutathione are known to be more resistant to the DNA damaging effects of X-rays (19,20). No DNA strand breaks were observed following treatment of cells for 1 hr with 100 μM $CdCl_2$, 500 μM Pb acetate, 500 μM $CoCl_2$, 1 mM $ZnCl_2$, 500 μM $CuSO_4$ and 100μM $NiCl_2$. Since intracellular effects of metals is highly dependent upon their ability to enter cells, we have examined the uptake of a variety of toxic metal ions (Cd^{2+}, Hg^{2+}, CrO_4^{-2}, Co^{2+}, Cu^{2+}, and Pb^{2+}) following 4 hr of exposure. The following exposure conditions yielded the indicated levels of metal associated with cells (ng/10^4 cells): $HgCl_2$ (50μM) = 3.55, $CdCl_2$ (50μM) = 0.35, $ZnCl_2$ (500μM) = 1.63, $CoCl_2$ (500 μM) = 0.54, $CuSO_4$ (500μM) = 0.28, Pb acetate (500μM) = 60.75, $CaCrO_4$ (100μM) = 1.7. Thus, Hg^{2+} appears to be the only toxicologically important metal ion that can cause rapid and pronounced breakage

of DNA similar to X-rays. This observation may be related to the high level of chemical reactivity of Hg^{2+} and to its pronounced cellular uptake compared with other metal ions (vide supra).

DISCUSSION

At acute cytotoxic concentrations the effects of Hg^{2+} resemble those produced by X-rays. These effects of Hg^{2+} have relevance since at less cytotoxic levels the X-ray-like effects may be occurring on a smaller scale, resulting in similar cellular perturbations as low doses of X-rays. The most serious of these long term effects may involve a mutagenic or carcinogenic action (7). The most striking evidence that Hg^{2+} produces X-ray like effects in cells includes: 1) the rapid and pronounced depletion of GSH which could result in cytotoxicity from enhanced oxygen radical formation. Both agents have been shown to enhance the formation of oxygen free radicals (13). 2) The induction of DNA strand breaks caused by both agents are similar in dose response, time course and alkaline elution kinetics (first order with respect to time); the latter indicating that Hg^{2+} results in a random distribution of single strand breaks in the DNA which is also produced by X-rays. 3) The lack of potentiation of DNA strand breaks by cytotoxic concentrations of both agents combined. 4) The inverse correlation between the cellular level of GSH and the induction of DNA strand breakage caused by both agents (19,20).

The rapid uptake of Hg^{2+} into cells combined with its high degree of chemical reactivity probably contributes substantially to its depletion of GSH. For example, of the toxicologically relevant metals, Hg^{2+} has been shown to possess a high degree of chemical softness (σ_p), a measure of the ability of a metal to form coordinate covalent bonds. However it is extremely interesting that while all the metal ions examined, including Cd^{2+}, possess a high degree of chemical reactivity, only Hg^{2+} produced X-ray like effects in cells. These X-ray like effects are probably responsible for the acute cytotoxicity of Hg^{2+} at the cellular level.

ACKNOWLEDGEMENTS

We thank Ms. Linda Haygood for secretarial assistance. This work was supported by Grant # R-808048 from the U.S. Environmental Protection Agency by

contract # DE-A505-81ER 60016 from the U.S. Dept. of Energy and by NIH Grant # CA 29581 from the National Cancer Institute and by research training grant # ES 07090 from the NIEHS. The EPA does not necessarily endorse any commercial products used in this study and the conclusions represent the views of the authors and do not represent the opinions, policies or recommendations of the EPA.

REFERENCES

1. Ramel, G. (1972) in Mercury in the Environment, 169-181.
2. Heck, J.D. and Costa, M. (1982) Biol. Trace Elem. Res. (in press).
3. Casto, B.C., Myers, J.D., and DiPaolo, J.A. (1979) Cancer Res. 39, 193-197.
4. Leonard, A. (1979) in Trace Metals, Exposure and Health Effects.
5. Miller, R. and Hall, E.J. (1978) Nature 272, 58-60.
6. Sobels, F.H. (1963) Radiation Res. Suppl. 3, 171-183.
7. Kaplan, H.S. and Brown, M.B. (1952) J. Natn. Cancer Inst. 13, 185-192.
8. Mills, M.D. and Meyn, R.E. (1981) Radiation Res., 87, 314-328.
9. Veatch, W. and Okada , S. (1969) Biophys. J. 9, 330-346.
10. Kohn, K.W., Ewig, R.A.G., Erikson, L.C. and Zwelling, L.A. (1981) in DNA Repair, a Laboratory Manual of Research Procedures, 379-401.
11. Kohn, K.W. (1979) in Methods in Cancer Research, Vol. 16, 291-345.
12. Stacey, N.H. and Kappus, H. (1982) Toxicol. Appl. Pharmacol. 63, 29-35.
13. Armel, P.R., Strinste, G.F. and Wallace, S.S. (1977) Radiation Res. 69, 328-338.
14. Bradley, M.O. and Erikson, L.C. (1981) Biochem. Biophys. Acta 654, 135-141.
15. Katz, S. (1963) Biochim. Biophys. Acta 68, 240-246.
16. Hissin, P.J. and Hilf, R. (1976) Anal. Biochem. 74, 214-218.
17. Dougle, D.L., Gillespie, G.J. and Chapman, J.D. (1976) Proc. Natl. Acad. Sci. USA 73, 809-812.
18. Costa, M., Simmons-Hansen, J., Bedrossian, G.W.M., Bonura, J., and Caprioli, R.M. (1981) Cancer Res., 41, 2868-2876.
19. Stankova, L., Rigas, D.A., Keown, P. and Bigley, R. (1977) J. Reticuloend. Soc. 2, 97-102.
20. Bump, E.A., Yu, N.Y. and Brown, J.M. (1982) Science 217, 544-545.