0026-895X/87/020200-08$02.00/0

MOLECULAR PHARMACOLOGY, 31:200-207

In Vivo Effects of Mercury (II) on Deoxyuridine Triphosphate Nucleotidohydrolase, DNA Polymerase (α, β), and Uracil-DNA Glycosylase Activities in Cultured Human Cells: Relationship to DNA Damage, DNA Repair, and Cytotoxicity

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Received August 12, 1986; Accepted November 25, 1986

SUMMARY

The effect of mercuric acetate on the activities of deoxyuridine triphosphate nucleotidohydrolase (dUTPase), DNA polymerase (α, β), and uracil-DNA glycosylase has been studied in cultured human KB cells. There was a dose- and time-dependent inactivation of both dUTPase and DNA polymerase α activities by mercuric acetate. In cells exposed to low concentrations (10 μM) of mercuric acetate, dUTPase was most sensitive to inhibition with 30% of the activity being inhibited after a 1-hr exposure. At higher concentrations or for longer exposure times, DNA polymerase α was most sensitive to inhibition with greater than 60% of the activity being inhibited by 25 μM mercuric acetate after a 15-min exposure. There was no inhibition of DNA polymerase β or uracil-DNA glycosylase activities in cells exposed to 50 μM mercuric acetate for 90 min. In fact, there was a time- and dose-dependent activation of uracil-DNA glycosylase activity with maximum activation occurring in cells exposed to 50 μM mercuric acetate. The inhibition of dUTPase and DNA polymerase α activities and the activation of uracil-DNA glycosylase activity correlated with the induction of single-strand breaks in DNA by mercuric acetate and with the decrease in cell viability.

Recent studies concerning the cytotoxicity of mercury (II) compounds in eukaryotic cells have focused on their effects on DNA biosynthesis and DNA repair (1–8). Mercury (II) compounds have been reported to cause an S-phase-specific cell block (3), to inhibit DNA synthesis (1, 2) and semiconservative replication (4), and to induce the formation of SSBs in DNA (5–7). Studies have also shown that, although mercury (II) compounds induce DNA repair in treated cells, the levels of DNA repair are reduced when compared to the levels of repair induced by metals, such as chromium and nickel, that possess carcinogenic activity (8). Conversely, concentrations of mercury (II) that produce relatively few SSBs in DNA have been reported to inhibit the repair of X-ray-induced SSBs (4, 6, 7), but not the repair of breaks induced by UV light (4). This has led to the hypothesis that mercury (II) compounds interfere with DNA homeostasis by inhibiting DNA repair processes and that this represents an irreversible injury that leads to cell death (7). However, none of the studies have examined what effect the *in vivo* exposure of cells to mercury (II) compounds has on the enzymes involved in DNA synthesis and repair.

The formation of dUMP residues in DNA either by the direct incorporation of dUTP by various DNA polymerases (9, 10) or by the spontaneous deamination of dCMP residues results in the transient formation of SSBs in DNA due to the activation of the uracil-DNA glycosylase base excision repair system (11, 12). Previous studies in our laboratory have demonstrated that various mercury (II) compounds act as active site-directed, irreversible inhibitors of eukaryotic dUTPase (EC 3.6.1.23), the enzyme responsible for hydrolyzing intracellular dUTP (13). The inhibition of dUTPase activity *in vivo* by mercury compounds could result in the increased incorporation of dUTP into DNA and the subsequent activation of the uracil-DNA glycosylase base excision repair system. The activation of this system could result in the generation of SSBs in DNA and possibly cell death depending on the level of dUTP incorporated into DNA. A similar phenomenom relating to the disruption of normal deoxyuridine metabolism has been proposed as a mechanism of cytotoxicity for specific antifolates (14, 15).

The results of this study demonstrate that exposure of human KB cells to mercuric acetate results in a dose- and time-

This work was supported in part by Grant DE-06866 from the National Institute for Dental Research (M. V. W.) and in part by Ohio State University Comprehensive Cancer Center Core Grant P30 CA 1605813 from the National Cancer Institute.

ABBREVIATIONS: SSB, single-strand break; dUTPase, deoxyuridine triphosphate nucleotidohydrolase; DMEM, Dulbecco's modified Eagle's medium; BSA, bovine serum albumin; TCA, trichloroacetic acid; PBS, phosphate-buffered saline; EDTA, ethylenediaminetetraacetic acid; SSF, strand scission factor; CHO, Chinese hamster ovary.

dependent inactivation of dUTPase and DNA polymerase α activities, but not DNA polymerase β or uracil-DNA glycosylase activities. In fact, there is a dose- and time-dependent activation of uracil-DNA glycosylase activity. The inhibition of dUTPase and DNA polymerase α activities, as well as the activation of uracil-DNA glycosylase, correlates with the induction of SSBs and the cytotoxicity of mercuric acetate.

Materials and Methods

Chemicals. Nonradioactive nucleoside triphosphates were purchased from Sigma Chemical Co., St. Louis, MO. Radiolabeled [5-^{3}H]dUTP (11 Ci/mmol) was purchased from Moravek Biochemicals, Brea, CA; [5-*methyl*-^{3}H]dTTP (63 Ci/mmol), [5-*methyl*-^{3}H]thymidine (71 Ci/mmol), and 3,4-[^{3}H]leucine (40 Ci/mmol) were purchased from ICN Radiochemicals, Richmond, CA. DMEM was purchased from Flow Laboratories, McLean, VA, and bovine serum was purchased from GIBCO, Grand Island, NY. Mercuric acetate and tetrapropylammonium hydroxide were obtained from Aldrich Chemical Co., Milwaukee, WI.

Cell culture procedures. Human KB cells were grown in DMEM containing 5% (v/v) bovine serum, 1% (v/v) nonessential amino acids, and gentamicin (50 μg/ml) (complete DMEM) in a 5% CO_2 atmosphere at 37°. Randomly growing cells were used in all studies. Prior to treatment with mercuric acetate, cells were collected by low speed centrifugation, washed in prewarmed serum-free DMEM, and then resuspended in serum-free DMEM.

Preparation of crude cell extracts for enzyme analysis. Following the exposure of cells to mercuric acetate, cells ($1–5 \times 10^6$) were washed three times with serum-free DMEM, collected by low speed centrifugation, and stored at −20° until use. Crude cell extracts were prepared by suspending the cells in 10 mM Tris-HCl, pH 8.0, containing 20% (v/v) glycerol, 2 mM 2-mercaptoethanol, 1 mM $MgCl_2$, and 0.2 mM phenylmethylsulfonyl fluoride. The suspension was frozen and thawed twice, followed by sonic oscillation using a Branson Sonifier, model 350 (four 15-sec bursts with the microtip setting 4). The resulting homogenate was centrifuged at 12,000 × *g* for 10 min at 4°. The resulting supernatant was used for the enzyme analyses. All extracts were assayed immediately for the enzymatic activities.

Enzyme assays. dUTPase activity was determined using the procedure described by Williams (16). The reaction mixture contained in a total volume of 0.1 ml: 50 mM Tris-HCl, pH 8.0, 2 mM 2-mercaptoethanol, 0.1% (w/v) BSA, 0.1 mM [^{3}H]dUTP (50 μCi/mmol), 1 mM $MgCl_2$, 0.2 mM *p*-nitrophenyl phosphate, and the cell extract (20–40 μg of protein). The reaction mixtures were incubated at 37°. Samples were removed at 15-min intervals for 1 hr and the reactions were terminated by spotting 50 μl of the reaction mixture on a DE-81 filter disc and immediately washing the disc in a solution of 4 M formic acid and 1 mM ammonium formate. Discs were then processed as described previously (16). A unit of dUTPase activity was defined as the amount of enzyme required to convert 1 nmol of dUTP to dUMP and pyrophosphate per min at 37°.

Uracil-DNA glycosylase activity was determined using a modification of the procedure described by Cardonna and Cheng (12). Double-stranded DNA containing [^{3}H]uracil residues was prepared from calf thymus DNA activated by DNase according to the method of Rigby *et al.* (17). The resulting gapped DNA was used as a template-initiator system in the reaction catalyzed by *Escherichia coli* DNA polymerase I (endonuclease free, Boehringer Mannheim), with a standard incubation mixture containing [^{3}H]dUTP in place of dTTP. The uracil-DNA glycosylase reaction mixture contained in a total volume of 0.2 ml: 50 mM Tris-HCl, pH 7.5, 2 mM dithiothreitol, 100 μg/ml of BSA, 3 μg of [^{3}H]uracil-labeled DNA, and the cell extract (20–40 μg of protein). The reaction mixtures were incubated at 37° for 1 hr with the reactions being terminated at 15-min intervals. Reactions were terminated by adding 25 μl of sheared calf thymus DNA (1 mg/ml) and 25 μl of cold 50% (w/v) TCA and then placing the mixture in ice for 15 min. The reaction mixtures were centrifuged at 1400 × *g* for 5 min, 0.2 ml of the supernatant was removed, and radioactivity was determined by scintillation counting. A unit of uracil-DNA glycosylase activity was defined as the amount of enzyme required to release 1 nmol of [^{3}H]uracil as acid-soluble material per min at 37°.

DNA polymerase α and β activities were determined using the assay procedures described by Ruth and Cheng (18). The DNA polymerase α reaction mixture contained in a total volume of 0.1 ml: 70 mM Tris-HCl, pH 8.0, 8 mM $MgCl_2$, 70 μg of BSA, 0.7 mM dithiothreitol, 2% (v/v) glycerol, dATP, dGTP, and dCTP (500 μM each), 10 μM [^{3}H]dTTP (50 μCi/mmol), 25 μg of activated calf thymus DNA, and the cell extract (20–40 μg of protein). The DNA polymerase β contained, in addition to those in the DNA polymerase reaction mixture, 100 mM KCl. Reaction mixtures were incubated at 37° for 1 hr and were terminated by spotting 50 μl of the mixture on a Whatman 3MM filter disc and immediately washing the disc in a cold solution of 5% (w/v) TCA and 1% (w/v) sodium pyrophosphate. The discs were washed twice in this solution for 10 min each and once in 95% ethanol. Discs were dried and the radioactivity bound to the disc was determined by scintillation counting. A unit of DNA polymerase activity was defined as the amount of enzyme which incorporated 1 pmol of [^{3}H]dTTP into acid-insoluble material per hr at 37°.

Protein determination. Protein was estimated using the Coomassie blue dye-binding assay as described by Bio-Rad Laboratories (Richmond, CA) using BSA as the standard.

DNA and protein synthesis. DNA synthesis was measured using a modification of the procedure described by Frenkel and Randles (19). Cells were grown in complete DMEM and collected by low speed centrifugation. Cells (1×10^6) were resuspended in serum-free DMEM and were treated with various concentrations of mercuric acetate while simultaneously being pulsed with [^{3}H]thymidine (3 μCi/ml) for 30 min at 37°. The cells were washed once with PBS and then resuspended in 1 ml of phosphate lysis buffer [PBS containing 0.1% (w/v) sodium dodecyl sulfate and 10 mM disodium EDTA]. Cold TCA [10% (w/v), 1 ml] was added to the lysate and the solution was incubated on ice for 30 min. The acid-insoluble material was collected on Gelman A/E glass fiber discs (Gelman Sciences Inc., Ann Arbor, MI). The discs were washed three times with cold PBS and dried, and acid-insoluble radioactivity bound to the disc was determined by scintillation counting.

Protein synthesis was measured using a modification of the procedure described by Gruenweld and Cruickshank (1). Cells (1×10^6) were exposed to mercuric acetate for various time periods in serum-free DMEM. Following the treatment cells were collected by centrifugation, washed in prewarmed leucine-deficient DMEM, and resuspended in the leucine-free DMEM. [^{3}H]Leucine (2.5 μCi/ml) was added to the cell suspension and the suspension was incubated at 37° for 30 min. Following the incubation, cells were collected by centrifugation and washed twice with cold PBS to remove unincorporated leucine. Cells were then resuspended in PBS and collected by filtration on 0.45-μm filters (Amicon Corp., Lexington, MA). Filters were washed with 3 ml of cold PBS followed by three 3-ml washes with cold 5% (w/v) TCA. Filters were dried and the acid-insoluble radioactivity bound to the filters was determined by scintillation counting.

Cytotoxicity assays. The cytotoxicity of mercuric acetate was determined by measuring cell survival. For cell survival studies cells were treated with various concentrations of mercuric acetate for 1 hr at 37° in serum-free DMEM. Cells were collected by low speed centrifugation and washed twice with serum-free DMEM. Cells were resuspended in complete DMEM and incubated at 37° for 24 hr. Viability was determined by trypan blue exclusion. Cell survival is expressed as the percentage of viable cells in treated cultures when compared to nontreated controls.

Alkaline elution. The alkaline elution technique for the analysis of DNA strand breaks was performed essentially as described by Kohn *et al.* (20). Cells (1×10^6) were grown in DMEM containing [^{3}H]thymidine (1.25 μCi/ml) for 24 hr at 37°. The cells were washed, resuspended in fresh DMEM, and incubated an additional 24 hr. Cells

were then removed from plates by scraping, washed in serum-free DMEM, and resuspended in serum-free DMEM for treatment with mercuric acetate. Following treatment, cells were washed with cold PBS and the cells (5×10^6) were deposited on 25-mm polycarbonate filters (Bio-Rad Laboratories). Filters were rinsed with 10 ml of cold PBS and the cells lysed directly on the filters by passage of 5 ml of 2% (w/v) sodium dodecyl sulfate (electrophoretic grade, Bio-Rad Laboratories) containing 0.025 M EDTA, pH 9.7. The DNA was eluted from the filter by pumping (0.027 ml/min) 25 ml of a solution containing 0.02 M EDTA (free acid) and 2% (v/v) tetrapropylammonium hydroxide, pH 12.7. Fractions were collected at approximately 75-min intervals. Following the completion of the elution procedure, the filters were digested for 1 hr at 60° in 1 M HCl. The amount of radioactivity in an 0.8-ml aliquot of each fraction was determined by scintillation counting using Budget Solve containing 0.7% glacial acetic acid.

To quantitate the extent of SSBs in DNA of mercury (II)-treated cells, the SSF was calculated from the alkaline elution profiles using the following relationship: SSF = log f_A/f_B, where f_A is the amount of DNA retained on the filter 5 hr after initiation of elution in the untreated control and f_B is the amount of DNA retained on the filter 5 hr after initiation of elution in the treated sample.

Results

Following the exposure of KB cells to mercuric acetate there was a time- and dose-dependent inhibition of both dUTPase (Fig. 1A) and DNA polymerase α (Fig. 1B) activities. A 10 μM concentration of mercuric acetate caused a 10% inhibition of dUTPase activity after 15-min exposure and the inhibition increased to 47% after a 90-min exposure. Maximum inhibition (85%) of dUTPase activity occurred in cells exposed to 50 μM mercuric acetate for 90 min.

There was no inhibition of DNA polymerase α activity in cells exposed to 5 μM mercuric acetate for 90 min or to 10 μM for 60 min. However, exposure of the cells for 90 min to 10 μM mercuric acetate resulted in a 62% inhibition of DNA polymerase α activity. Maximum inhibition of DNA polymerase α activity (90%) occurred in cells exposed to 50 μM. There was no inhibition of uracil-DNA glycosylase (Fig. 1C) or DNA polymerase β (Fig. 1D) activities in cells exposed for 90 min to mercuric acetate concentrations up to 50 μM. In fact, mercuric acetate stimulated uracil-DNA glycosylase activity in a dose- and time-dependent manner but not DNA polymerase β activity. Maximum stimulation of uracil-DNA glycosylase activity occurred in cells exposed for 15 min to 50 μM mercuric acetate. This represented a 1.3-fold increase in the level of the enzyme activity in treated cells (0.20 unit/mg of protein in treated cells

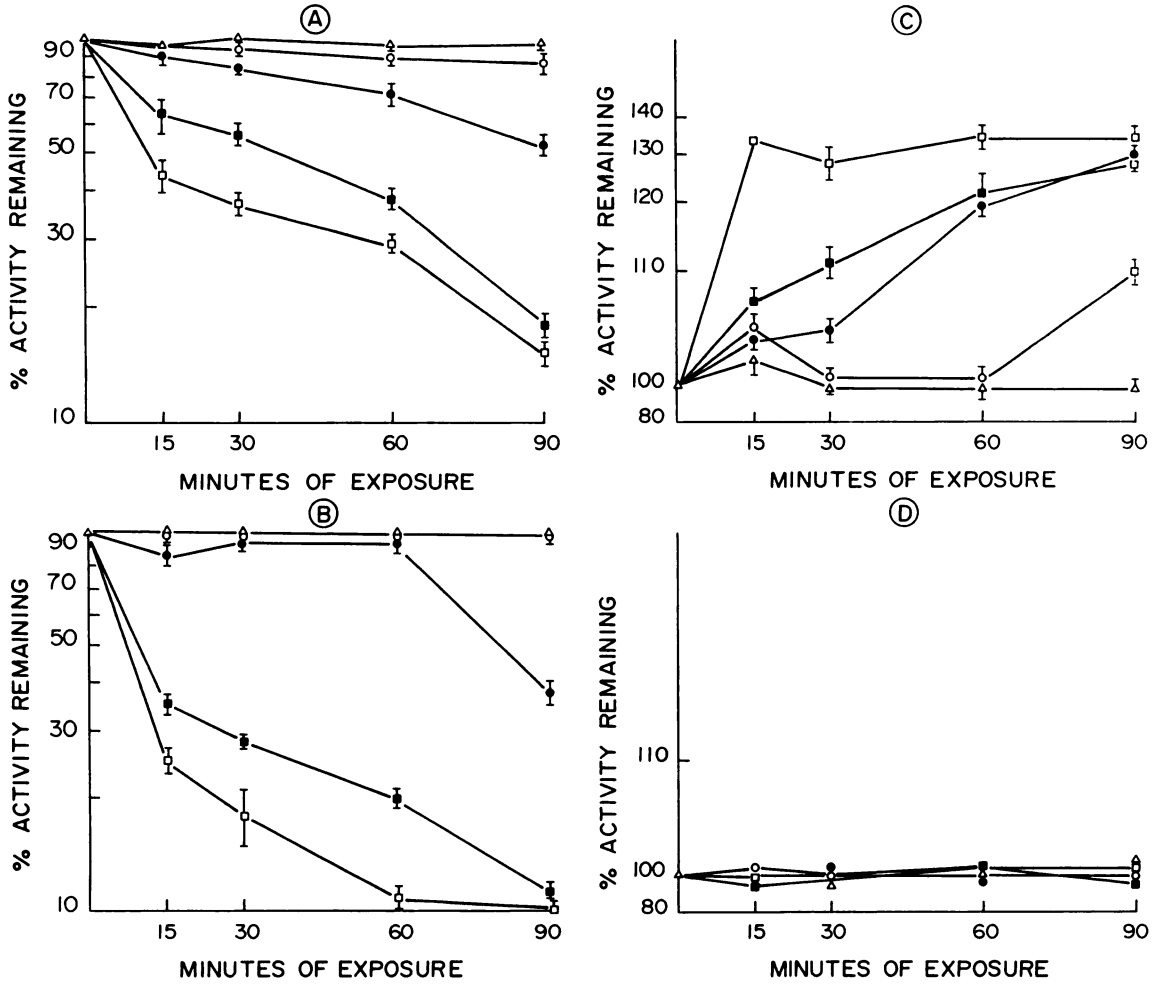

Fig. 1. Effect of mercuric acetate on dUTPase, DNA polymerase (α,β), and uracil-DNA glycosylase activities in KB cells. Intact cells were exposed to various concentrations of mercuric acetate for the times indicated. Cell extracts were prepared and enzymatic assays were performed as described in Materials and Methods. Extracts were assayed simultaneously for the various enzymes. The values represent the average ± the standard deviation for three experiments. A, dUTPase; B, DNA polymerase α; C, uracil-DNA glycosylase; D, DNA polymerase β. △, untreated; ○, 5 μM; ●, 10 μM; ■, 25 μM; □, 50 μM mercuric acetate.

versus 0.15 unit/mg in controls). Longer exposure times of the cells to this concentration of mercuric acetate did not further stimulate or inhibit the levels of uracil-DNA glycosylase activity.

To determine whether the changes in the levels of these enzymes were due to an *in vivo* effect or whether they were due to the presence of "unreacted" mercury (II) in the extracts which inhibited or activated the enzymes *in vitro*, mixing experiments were performed (Table 1). Mercuric acetate (5 μM), when added to the purified KB dUTPase, resulted in a 40% inhibition of the dUTPase activity. However, the crude extracts from cells exposed to mercuric acetate (50 μM) caused no inhibition of the purified dUTPase activity. Similarly, the same crude extracts did not cause any significant inhibition of DNA polymerase α activity (data not shown).

Exposure of KB cells to mercuric acetate also resulted in a dose-dependent decrease in cell viability (Table 2). Concentrations of 5 and 10 μM mercuric acetate reduced cell viability by 10 and 24%, respectively, whereas higher concentrations resulted in a greater than 80% reduction in viability. The IC_{50} (concentration required to inhibit 50% of cell viability after a 1-hr exposure to mercuric acetate) was estimated to be 13.9 μM. The decrease in cellular viability following a 1-hr exposure to mercuric acetate exhibited a direct correlation with the inhibition of dUTPase ($r = 0.992$) and DNA polymerase α ($r = 0.991$) activities and an inverse correlation with uracil-DNA glycosylase activity ($r = -0.880$). That is, the greater the activation of uracil-DNA glycosylase activity, the greater the decrease in viability.

The effect of mercuric acetate on protein synthesis (Fig. 2) and DNA synthesis (Fig. 3) was also examined. Protein synthesis, as measured by the incorporation of [^{3}H]leucine into acid-insoluble material, was inhibited in a dose- and time-dependent manner. The greatest inhibition (85%) occurred following the exposure of cells to 50 μM mercuric acetate for 1 hr. Conversely, DNA synthesis was stimulated slightly in cells exposed for 30 min to low concentrations (5 and 10 μM) of

TABLE 1

Mixing experiments

Inhibition studies were performed as described previously (13). The crude extracts were from cells exposed to 50 μM mercuric acetate for the times indicated. The units of dUTPase activity per ml of the extracts were 4.00, 1.80, 1.54, 1.20, and 0.60 for the 0-, 15-, 30-, 60-, and 90-min extracts, respectively. The additive (10 μl) was mixed with the purified KB dUTPase (0.02 unit) and incubated for 1 hr at 37°. Residual dUTPase activity was then determined by further incubation for 1 hr at 37° in a dUTPase reaction mixture that contained 40 μM [^{3}H]dUTP (13).

Additive	nmol of dUTP hydrolyzed	
	Expected	Observed[a]
	1.20	1.22 ± 0.04
Mercuric acetate[b]		0.73 ± 0.03
Crude extract, 0 min	3.60	3.62 ± 0.04
Crude extract, 15 min	2.28	2.28 ± 0.06
Crude extract, 30 min	2.12	2.15 ± 0.01
Crude extract, 60 min	1.92	2.01 ± 0.04
Crude extract, 90 min	1.56	1.58 ± 0.05

[a] Values represent the average of three experiments ± the standard deviation.
[b] The mercuric acetate concentration was 5 μM.

TABLE 2

Effect of mercuric acetate on KB cell viability

Mercuric acetate (μM)	Viability[a]
	% of control
5	90 ± 5.1
10	76 ± 7.4
25	13 ± 3.4
50	7 ± 2.6

[a] Approximately 2.5 × 10^5 cells were exposed to mercuric acetate for 1 hr in serum-free DMEM. Cells were then washed and resuspended in complete DMEM and incubated for an additional 24 hr. Viability was determined as described in Materials and Methods. The values represent the average ± the standard deviation of three experiments.

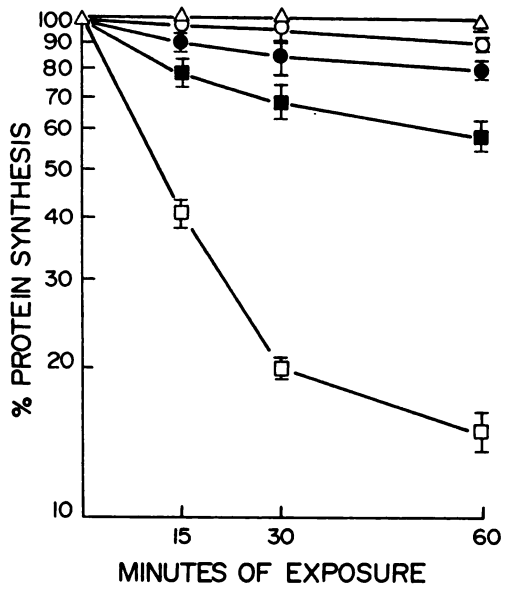

Fig. 2. Effect of mercuric acetate on protein synthesis. Cells were preexposed to various concentrations of mercuric acetate for the times indicated and protein synthesis was then determined as described in Materials and Methods. The values represent the average of at least two experiments ± the standard deviation. [^{3}H]Leucine incorporation in untreated controls was 55,563 ± 2,351 cpm. *Symbols* are as in Fig. 1.

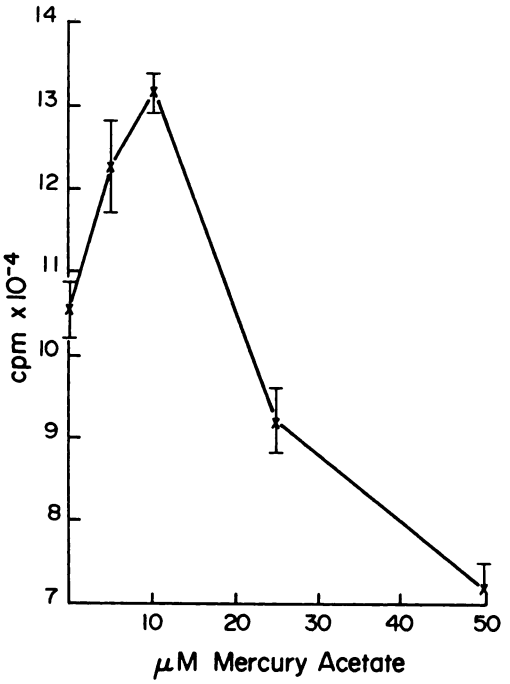

Fig. 3. Effect of mercuric acetate on DNA synthesis. Cells were exposed to various concentrations of mercuric acetate for 30 min and simultaneously pulsed with [^{3}H]thymidine. DNA synthesis was determined as described in Materials and Methods. The values represent the average ± the standard deviation of at least two experiments.

mercuric acetate and inhibited 13 and 32%, respectively, in cells exposed to 25 and 50 μM mercuric acetate.

To determine the relationship between the inhibition and/or activation of these enzymes and the induction of SSBs in DNA of cells exposed to mercuric acetate, alkaline elution analysis was performed (Fig. 4). Since alkaline-sensitive apyrimidinic sites as well as SSBs can be formed in DNA due to the action of uracil-DNA glycosylase base excision repair system (11, 12), alkaline elution analysis was performed at a pH of 12.7. At this pH both SSBs and alkaline-sensitive sites in DNA would be measured (20). There was a time- and dose-dependent induction of SSBs in cells exposed to mercuric acetate as reflected by the increase in the SSF (Fig. 5). Maximum DNA damage occurred in cells exposed to 50 μM mercuric acetate for 90 min (Fig. 5A). However, exposure of cells to mercuric acetate concentrations between 5 and 25 μM resulted in a biphasic response with respect to time of exposure. Initial DNA damage plateaued between 30 and 60 min exposure, but lengthening the exposure time to 90 min resulted in a dramatic increase in the level of DNA damage (Fig. 5B). There was a direct correlation between the induction of DNA damage in cells exposed to mercuric acetate for 1 hr and the inhibition of dUTPase ($r = 0.911$) and DNA polymerase α ($r = 0.867$) activities. The greater the inhibition of these enzyme activities the greater the amount of DNA damage. Furthermore, there was also direct correlation between the activation of uracil-DNA glycosylase activity ($r = 0.899$) and the induction of DNA damage. That is, the greater the activation of the enzyme, the greater the DNA damage.

Discussion

Mercury (II) is a highly reactive molecule that forms covalent bonds with a variety of cellular macromolecules (21). Although mercury (II) binds primarily to sulfhydryl-containing macromolecules, it can also bind to other non-sulfhydryl-containing macromolecules such as DNA (22–24).

Studies in cultured eukaryotic cells have demonstrated that organic and inorganic mercury (II) compounds in the concentration range of 0.5–200 μM have diverse effects on cellular function (1–8, 19, 25–28). Depending upon exposure times and cell types, low concentrations (10 μM or less) of mercury (II) compounds have been reported to have little effect on cellular viability and to stimulate RNA and DNA synthesis, whereas higher concentrations are cytotoxic and inhibit DNA, RNA, and protein synthesis (1, 2, 19, 25–28). Recent studies have demonstrated that acutely cytotoxic concentrations of mercuric chloride (5–100 μM) induce the formation of SSBs in DNA of CHO cells and that the induction of these SSBs resemble those induced by X-rays (4, 6, 7). These SSBs are frank breaks and are not due to the production of alkaline-sensitive sites, and they are not double-strand breaks (23, 24, 26). The induction of these SSBs is thought to involve the depletion of cellular glutathione levels and the production of oxygen radicals which

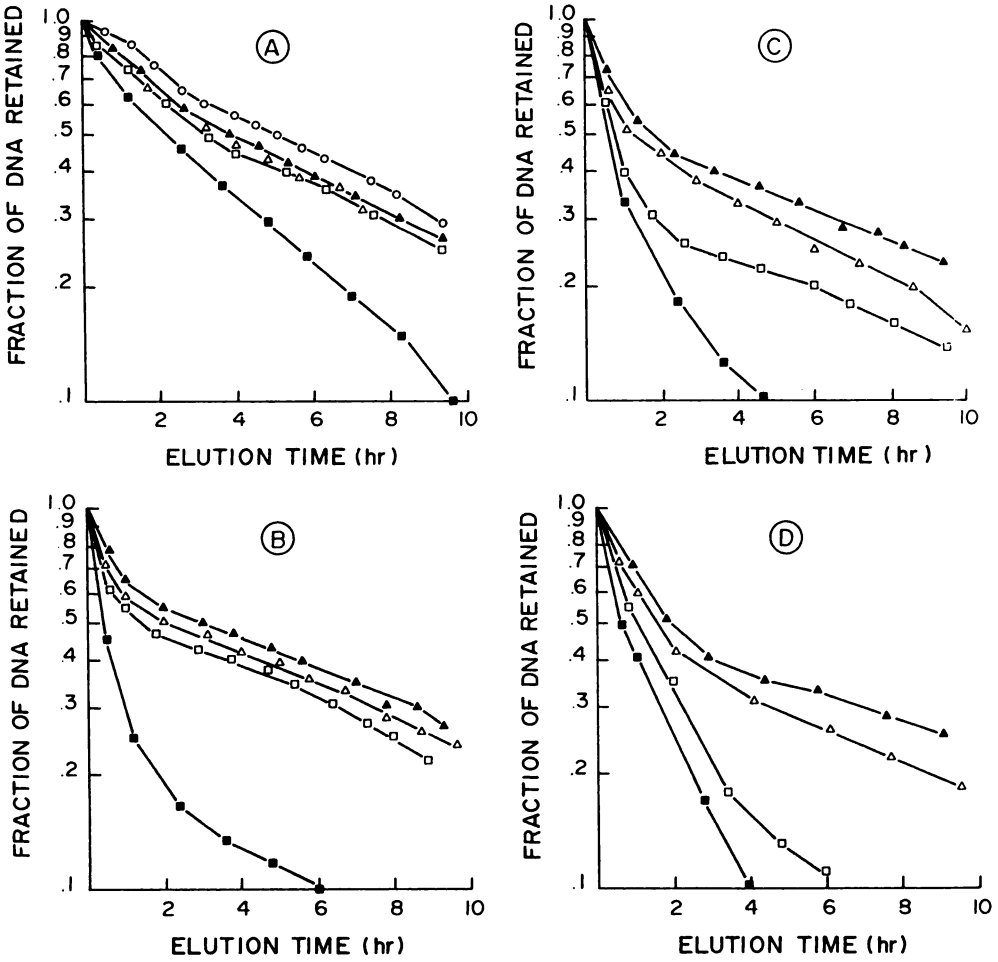

Fig. 4. Induction of SSBs in DNA by mercuric acetate. Alkaline elution analysis of DNA was performed as described in Materials and Methods. Values represent the average of at least two experiments. A, 5 μM mercuric acetate; B, 10 μM mercuric acetate; C, 25 μM mercuric acetate; D, 50 μM mercuric acetate. For simplicity, the untreated controls ○ are shown only in A. Mercury (II) exposure was: ▲, 15 min; △, 30 min; □, 60 min; and ■, 90 min.

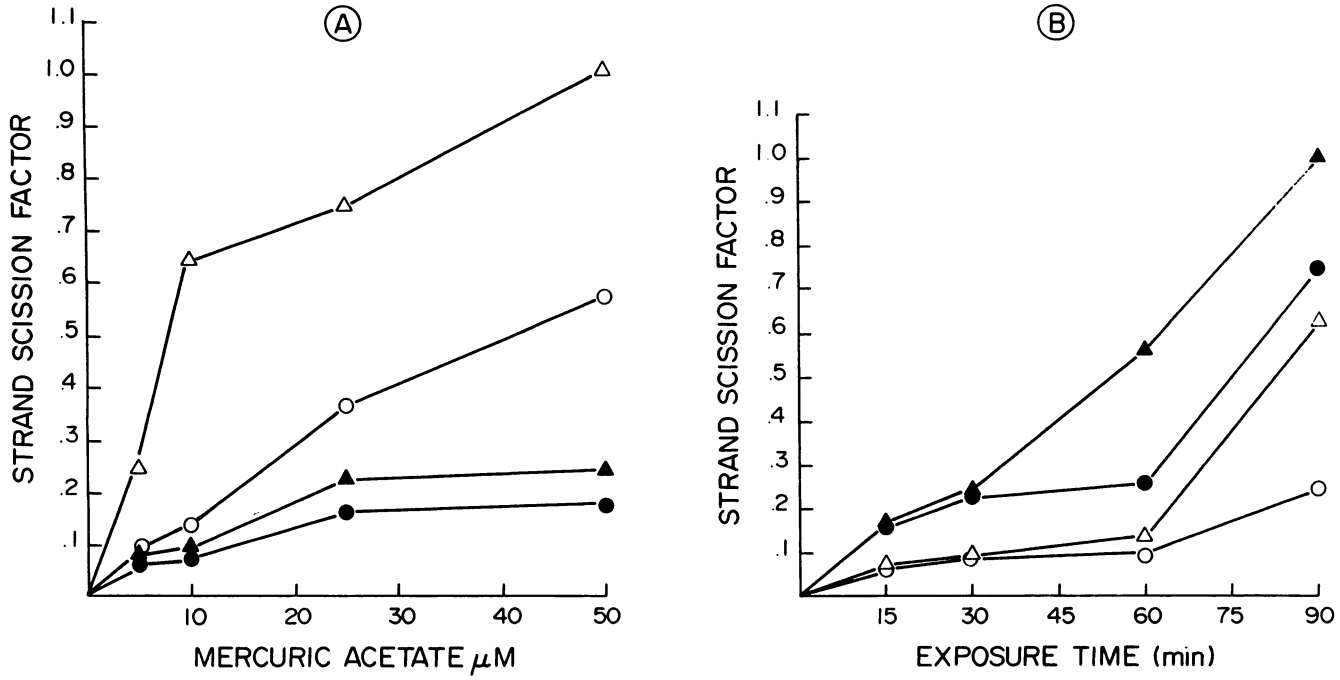

Fig. 5. Concentration- and time-dependent formation of SSBs in DNA exposed to mercuric acetate. The SSF was determined from the alkaline elution profiles as described in Materials and Methods. A. Concentration-dependent formation of SSB. ●, 15 min; ▲, 30 min; ○, 60 min; △, 90 min. B. Time-dependent formation of SSB. ○, 5 μM; △, 10 μM; ●, 25 μM; ▲, 50 μM.

ultimately damage the DNA (4, 6, 7, 23). Studies have also demonstrated in CHO cells that low concentrations (<20 μM) of mercuric chloride only induce 10% of the DNA repair activity that is induced by other metals which are mutagenic and/or carcinogenic (8), and that higher concentrations of mercury (II) inhibit the induction of DNA repair processes (4, 6–8, 23). Similarly, concentrations of mecuric chloride that do not induce significant levels of SSBs in DNA inhibit the repair of X-ray-induced SSBs (4, 6, 7). This has led to the proposal that mercury (II) inhibits some aspect of DNA repair and that this ultimately results in cell death (7, 23). This hypothesis has also been used to explain the weakly mutagenic properties of mercury (II) since it is thought that repair processes are a requirement for some types of mutagenesis (4, 6–8). However, none of these studies have determined what effect mercury (II) has on the activities of the various enzymes involved with DNA synthesis and repair processes.

The results of this study demonstrate the complex effects that mercury (II) has on enzymes involved in DNA synthesis and repair processes in human cells. In cells exposed for 1 hr or less to low concentrations of mercuric acetate (10 μM or less), dUTPase was most sensitive to inhibition. However, if the cells were exposed for longer time periods or to higher concentrations of mercury (II), DNA polymerase α was the enzyme most sensitive to inhibition. Conversely, mercuric acetate in concentrations up to 50 μM did not inhibit *in vivo* DNA polymerase β or uracil-DNA glycosylase activities. In fact, uracil-DNA glycosylase was activated in treated cells.

The results of our mixing experiments demonstrated that the inhibition of dUTPase and DNA polymerase α activities was not due to the presence of increased mercury (II) levels in extracts from treated cells. This suggests that the changes in enzyme levels must be due to some *in vivo* effect of mercury (II) such as the direct inhibition of the enzymes or the inhibition of some process involved in the synthesis of the enzymes. The direct inhibition of the enzymes is supported by studies using purified enzymes which demonstrated that mercury (II) compounds acted as active site irreversible inhibitors of eukaryotic dUTPases (13) and various DNA polymerases (2, 29). Although our studies suggest that the decrease in the levels of the enzymes cannot be explained by the decrease in protein synthesis, we cannot eliminate the possibility that the decrease in the levels of these enzymes is due to some selective alterations in either transcription or translation processes.

The inhibition of DNA polymerase α by mercury (II) would have diverse effects in a cell since this enzyme is involved in both DNA replication (30) and repair processes (31). Our data demonstrate that there is a direct correlation between the inhibition of DNA polymerase α activity and the inhibition of [^{3}H]thymidine incorporation into DNA. Although we did not examine DNA repair systems besides the uracil-DNA glycosylase base excision repair system, the repair of DNA damaged by agents such as X-rays, UV light, and certain chemicals that involve DNA polymerase α activity should also be reduced in cells exposed to mercury (II). Conversely, those repair systems that utilize DNA polymerase β would not be affected by mercury (II).

In addition to inhibiting DNA polymerase α, the results of this study also suggest that mercury (II) disrupts normal deoxyuridine metabolism in KB cells by inhibiting dUTPase activity. This inhibition could result in an increase in intracellular dUTP pools and thus an alteration in the normal dUTP/dTTP ratio. This would allow for the greater incorporation of dUTP into DNA and thus explain the activation of uracil-DNA glycosylase activity in cells exposed to mercury (II). This is further suggested in that there is a direct correlation between the

inhibition of dUTPase activity and the activation of uracil-DNA glycosylase activity with the induction of SSBs in the DNA of mercury (II)-treated cells. However, we have not been able to demonstrate any incorporation of [5-^{3}H]deoxyuridine into DNA of KB cells exposed to mercuric acetate, even in the presence of uracil (10 μM), a potent inhibitor of uracil-DNA glycosylase (data not shown). This could be due to the dilution of radiolabeled dUrd by endogenous dUrd which has been reported to increase following disruption of deoxypyrimidine metabolism (32), the loss of radiolabel due to the formation of mercurideoxyuridine derivatives (29), the effective removal of dUMP residues from DNA by uracil-DNA glycosylase even in the presence of uracil, the insensitivity of our methods for the detection of dUMP in DNA, the lack of dUTP incorporation into DNA, or a combination of the above. Whereas studies in cultured lymphocytes have demonstrated the incorporation of dUMP from deoxyuridine following treatment with antifolates (14, 15), a recent study on a number of cell lines exposed to methotrexate could not demonstrate, using very rigorous techniques, any incorporation of dUMP from deoxyuridine into the DNA (33). It is not known whether this reflects a quantitative difference between the level of deoxyuridine incorporation or differences in the efficiency of removal of dUMP from DNA in these cells.

Although we could not demonstrate the incorporation of deoxyuridine into DNA of KB cells exposed to mercury (II), the results of this study demonstrated that mercury (II) is very effective in inhibiting both dUTPase and DNA polymerase α activities at concentrations below that required to deplete glutathione levels (6) and in inhibiting those enzymes involved in glutathione metabolism (34). Studies in CHO cells demonstrated that the DNA damage observed in mercury (II)-treated cells is not due to the induction of alkaline-sensitive sites but rather to the induction of frank SSBs (23, 24, 26), and it has been proposed that the induction of these breaks involves both free radical formation and various enzymatic systems (23). Our studies in human cells corroborate these results since our alkaline elution profiles of DNA from mercury (II)-treated cells do not suggest the presence of alkaline-sensitive sites. Thus, it is possible that the DNA damage caused by mercury (II) is due to several different biochemical mechanisms. At low mercury concentrations the SSBs could be formed by the removal of dUTP by the uracil-DNA glycosylase base excision repair system. At higher concentrations additional damage in the form of SSBs and DNA-DNA crosslinks could be due to the generation of oxygen radicals (4, 6, 23) and the direct binding of mercury (II) to DNA (22–24).

The cytotoxicity of mercury (II) compounds is probably related to their ability to inhibit DNA polymerase α activity and, thus, to inhibit not only DNA synthesis but also DNA repair processes that utilize this enzyme as part of the repair system. Studies in CHO cells suggest that DNA replication is more sensitive to mercury (II) inhibition than is repair synthesis (4). However, the ability of a cell to survive exposure to relatively low doses of mercury (II) would be dependent upon differences in the rate at which DNA damage occurs and the rate at which repair occurs. By decreasing intracellular levels of DNA polymerase α, both directly and indirectly due to inhibition of protein synthesis, the rate of DNA repair would be decreased. This would result in greater DNA fragmentation and, ultimately, cell death. This is suggested by our results which demonstrate that there is an increase in the rate of induction of SSBs in cells exposed to low doses of mercuric acetate (10 μM) for an extended time (90 min) when compared to the rate of SSB induction in cells exposed to the same concentration of mercuric acetate, but for shorter time periods. This increase in SSB induction correlates with the inhibition of DNA polymerase α activity.

The weakly mutagenic properties of mercury (II) could be related to the incorporation of dUTP into DNA and the activation of the uracil-DNA glycosylase base excision repair system. The activation of this repair system has been demonstrated to be mutagenic in bacteria (35). It has been reported that mercury (II) is not mutagenic in *E. coli* test systems (36, 37) and the *E. coli* dUTPase is not inhibited by a number of mercury (II) compounds (13, 38). However, mercury (II) compounds have been reported to be mutagenic in *Bacillus subtilis* test systems (37) and the dUTPase from this organism has been reported to be inhibited by mercury (II) (39). The activation of this repair system may also explain the apparent increase in SSBs following exposure of cells to X-ray and nontoxic concentrations of mercury (II) (4, 6, 7), since alkaline elution techniques are not adequate for distinguishing this type of repair process from DNA damage.

This study demonstrates that mercury (II) can inhibit or activate enzymes involved in both DNA synthesis and repair processes and that this would have diverse effects on cellular function. However, it is also apparent from the studies that further research is needed to determine what effect low doses of mercury (II) have on deoxyribonucleotide pools, the effect that mercury (II) has on other enzymes involved in DNA repair processes, and the relationship between mutagenicity and the inhibition or activation of these repair systems, especially the uracil-DNA glycosylase base excision repair system.

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