0026-895X/87/010021-06$02.00/0

MOLECULAR PHARMACOLOGY, 31:21-26

Induction of Metallothionein is Correlated with Resistance to Auranofin, a Gold Compound, in Chinese Hamster Ovary Cells

BRETT P. MONIA,[1] TAUSEEF R. BUTT, CHRISTOPHER K. MIRABELLI, DAVID J. ECKER, EDMUND STERNBERG, and STANLEY T. CROOKE

University of Pennsylvania, Department of Pharmacology, Philadelphia, Pennsylvania 19104 (B.P.M., C.K.M., S.T.C.) and Smith Kline and French Laboratories, Department of Molecular Pharmacology, Philadelphia, Pennsylvania 19101 (T.R.B., C.K.M., D.J.E., E.S., S.T.C.)

Received April 24, 1986; Accepted October 7, 1986

SUMMARY

Metallothioneins (MTs) are low molecular weight, thiol-rich, metal-binding proteins. Auranofin (AF) is a gold compound active in the treatment of rheumatoid arthritis. The effects of AF on regulation of MT gene expression in Chinese hamster ovary cells were studied. AF-resistant cells accumulated substantial amounts of MT mRNA and protein, whereas no induction was observed in AF-sensitive cells. Cells capable of inducing MT in the presence of AF were much less sensitive to AF-mediated cytotoxicity. Induction of MT by low concentrations of Cd protected cells from subsequently administered doses of AF. The level of protection correlated with the level of induced MT. These findings indicate that MT plays a central role in the mechanisms underlying cellular resistance to gold compounds.

AF, (1-thio-β-D-glucopyranose 2,3,4,6-tetraacetato-*S*) (triethylphosphine)gold (Fig. 1), is an orally absorbed gold compound active in the treatment of rheumatoid arthritis (1–3). Current evidence suggests that an important therapeutic target of the drug is the macrophage, which plays a critical role in the etiology of rheumatoid arthritis (4). It has been shown that AF alters suppressor macrophage function by retarding the production of IL-2 (4). More recent studies in our laboratory indicate that there exists a dynamic equilibrium of the drug between the cell membrane, the cytoplasm, and the nucleus (5). A model describing the influx and efflux of the drug in tissue culture cells which involves shuttling of the gold molecule between various sulfhydryl-containing cellular moieties has been proposed (5). Furthermore, it is very likely that the active moiety of AF is the gold atom, which interacts with sulfhydryl-containing proteins. Gold may modulate the activities of certain enzymes by replacing a metal cofactor, by blocking the active site, or by causing conformational changes. For example, it has been reported that gold compounds inhibit DNA polymerase α, a sulfhydryl-containing enzyme, but not DNA polymerase β (6).

AF has also been shown to have antitumor activity in mice inoculated with the lymphocytic leukemia, P388 (7, 8), but limited activity in other tumor models (8). A number of other gold compounds have been shown to have more significant antitumor activities *in vitro* and *in vivo* (9). Mechanisms by which gold compounds induce the cytotoxic effect have not been defined.

One site at which gold-containing drugs may interact is with the sulfhydryl-rich, small molecular weight proteins, e.g., MTs (10). MTs are evolutionarily conserved proteins, distributed throughout mammals and lower eukaryotes (10–13). MTs are thought to play a role in heavy metal detoxification and zinc homeostasis (10). Mammalian cells contain two forms of MT designated as MT-I and MT-II. In humans, MTs are encoded by a complex gene family (13). Heavy metals such as Cd, Zn, Cu, Ag, Co, Hg, and Bi elevate MT levels by increasing the transcription of the MT genes (14). In addition to heavy metals, glucocorticoids and interferons also regulate MT gene expression (14–17). DNA sequences responsible for the Cd-mediated increase in transcription have been identified in the mouse MT-I and human MT-IIA genes (16, 18). The DNA sequences required for Cd-induced transcription appear to be different from those responsible for glucocorticoid-mediated gene activation of human MT-IA genes (16).

A number of reports document the interactions of gold molecules with MTs (19–22). More recently, we have demonstrated that gold is a potent inducer of MT gene transcription in CHO-AU^r cells in tissue culture. The extent of MT induction is related to the concentration of the compound and is affected by the nature of the ligand(s) attached to the gold atom (23).

[1] Predoctoral trainee supported by National Institutes of Health Grant GM-08076.

ABBREVIATIONS: AF, auranofin; MT, metallothionein; CHO-Au^r, Chinese hamster ovary cells resistant to auranofin; CHO, Chinese hamster ovary cell; CHO-Cd^r, Chinese hamster ovary cells resistant to cadmium; CHO-WT, wild-type Chinese hamster ovary cell; PBS, phosphate-buffered saline; EDTA, ethylenediaminetetraacetic acid.

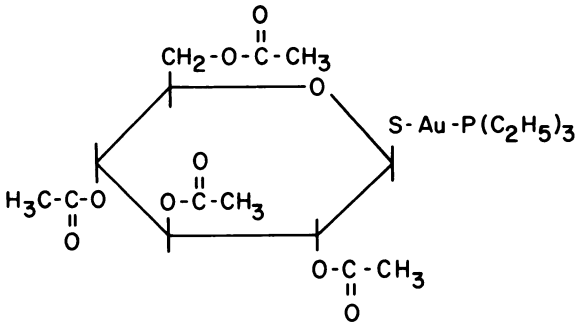

Fig. 1. Structure of gold-based drug AF. 1-Thio-β-D-glucopyranose 2,3,4,6-tetraacetato-*S*-(triethylphosphine)gold.

In addition, the Au-MT displays a much shorter *in vivo* half-life than Zn or Cd-MT in these cells grown in tissue culture (24).

Studies performed by Dinn and Frazier (25) have shown that animals or primary tissue culture cells can be protected from Cd toxicity by prior administration of Zn. The mechanism of protection in these studies has been attributed to prior induction of MT by Zn, resulting in sequestering of cellular Cd by MT and thus preventing Cd from reaching target sites where it exerts its cytotoxic effects. It is not known whether Cd displaces the MT-bound Zn or whether Zn induces more MT than is required to bind all of the free Zn in the cell, thus generating a subsaturated MT available to bind Cd. Indeed, previous studies indicate that MT molecules containing a mixture of Cd, Zn, and Cu are found in cells (26).

In the present work, we have studied the role of MT in resistance to gold compounds. The ability of AF to induce MT accumulation in AF-sensitive CHO and CHO-Au^r cells and the protective effect of preinduced MT on AF-mediated cytotoxicity have been analyzed.We have shown that cells capable of inducing MT in the presence of AF are much less sensitive to AF-mediated cytotoxicity and that preinduced MT protects CHO cells from AF-mediated cytotoxicity. The level of protection correlates well with the level of preinduced MT. These results indicate that MT plays a central role in the mechanisms underlying the resistance to gold compounds.

Materials and Methods

Chemicals. Analytical grade chemicals were used throughout this study. Cadmium chloride and zinc sulfate (Gold-label) were obtained from Aldrich Chemical Co. (1-Thio-β-D-glucopyranose 2,3,4,6-tetraacetato-*S*)(triethylphosphine)gold (AF) was synthesized at Smith Kline and French Laboratories (1). Stock solutions (1–10 mM) of AF were prepared in 95% ethanol or dimethyl sulfoxide. [^{35}S]Cysteine was purchased from New England Nuclear. Diethylpyrocarbonate was purchased from Sigma Chemical Co.

Cell culture. CHO cells Cd^{r}20F4 (27) (CHO-Cd^r) are resistant to 20 μM $CdCl_2$ in continuous culture conditions and possess a 6-fold amplification of their MT genes as compared to CHO-WT which contain a single copy of MT-I and MT-II genes (28). These cells were subcultured in the absence of Cd for several months. Induction of MT gene transcription by AF was performed on cells maintained in the absence of Cd. AF- and Cd-resistant CHO cell lines were maintained on monolayer cultures with Ham's F-10 medium, supplemented with 15% newborn calf serum (Gibco). CHO-WT and Cd-resistant CHO cells were kindly provided by Dr. E. C. Hildebrand of Los Alamos National Laboratory (27). CHO-Au^r cells were developed as previously described (23).

Cell survival assay. Asynchronous populations of cells were harvested by trypsinization. The cells were then washed with medium and counted. The cells were diluted to a density of 1×10^5/ml, and 0.5 ml of cells was incubated at 37° in Ham's F-10 medium and inoculated with drug for 1 hr. After incubation, the cells were diluted to a density of 1000 cells/ml and 5 ml of cells (5000 cells) were plated on 60 × 15 mm tissue culture plates. Plates were incubated for 4–6 days at 37° in a CO_2 (5%) incubator. Thereafter, the medium was removed, and the plates were washed with 0.15 M NaCl, fixed, and stained with 1% crystal violet; colonies were counted by New Brunswick Automatic Colony Counter (New Brunswick, NJ). Means and standard deviations of triplicate samples were determined for each drug concentration. Viability was measured by the ability of cells to form colonies. Percentage of survival is equal to the number of colonies in the drug-treated plates per number of colonies in control.

Priming assays. Asynchronous populations of CHO-Cd^r cells were harvested by trypsinization. The cells were then washed with medium and counted. The cells were diluted to a density of 1000 cell/ml; 5 ml of cells (5000 cells) were then plated on 60 × 15 mm tissue culture plates in Ham's F-10 medium, supplemented with 15% newborn calf serum (Gibco), and incubated at 37° in a CO_2 (5%) incubator for 24–36 hr. Cells were then exposed to the indicated concentrations of Cd for 3 hr (primed) and washed three times with PBS. Although cells were protected significantly from AF cytotoxicity when primed with Cd for time periods between 1 and 5 hr, 3 hr priming gave maximal protection and hence was used for further priming studies. Primed cells were then challenged with the indicated concentrations of AF for 1 hr, washed three times with PBS, and incubated with fresh culture medium at 37° for 4–6 days. Thereafter, the medium was removed; plates were washed with 0.15 M NaCl, fixed, and stained; and colonies were measured as described above. Means and standard deviations of triplicate samples were determined for each drug concentration per experiment, and experiments were performed in triplicate.

Analysis of MT. Monolayers of CHO-WT, CHO-Cd^r, or CHO-Au^r cells were subcultured to semilogarithmic stages and incubated with 100 μCi of [^{35}S]cysteine (NEG 002T, New England Nuclear) for 8 hr. At this time, cells were challenged with drug for 3 hr in the presence of [^{35}S]cysteine (conditions identical to those described under Priming Assays). Cells were then washed with PBS and harvested. Approximately 1×10^6 cells were labeled with [^{35}S]cysteine per data point. Cells were pelleted in 1.5-ml Eppendorf Microfuge tubes and lysed in 200 μl of 20 mM Tris-HCl (pH 8), 10 mM β-mercaptoethanol, 0.4% Nonidet P-40, and 1 mM phenylmethylsulfonyl fluoride (lysis buffer). The lysate was centrifuged for 5 min in a microcentrifuge (Beckman Microfuge) to pellet the debris, and supernatant was recovered for further analysis.

Equal amounts of protein (100 μg) were aliquoted from each sample supernatant and subjected to 18% polyacrylamide gel electrophoresis in the absence of urea or sodium dodecyl sulfate (12). The gel was fixed in acetic acid, treated with Autofluor (National Diagnostics), dried, and autoradiographed. Gels were exposed for 2, 4, and 6 days and each autoradiograph was quantified by scanning on a Beckman DU8 gel scanner. Bands corresponding to MI-I and MT-II were scanned separately and their averages were determined. Values represent means ± standard deviations for three experiments.

Analysis of mRNA. The effect of the test compounds on the regulation of MT mRNA transcription was analyzed by Northern blot analysis as described previously (11, 29). Briefly, for the preparation of total RNA, cells (5×10^6) grown in T-75 flasks were challenged with various compounds for appropriate periods of time. Cyclohexamide was added to the cultures (50 μg/ml) 5 min before harvesting (30). The cell layer was washed with 5 ml of PBS and suspended in 5 ml of PBS. Cells were pelleted, washed, and lysed in 0.50 ml of 10 mM Tris-HCl, pH 8, 1 mM EDTA, 1X vanadium ribonucleoside complex (Bethesda Research Laboratories, Rockville, MD), and 0.4% Nonidet P-40 for 10 min at 4°. The lysate was centrifuged in a table top microcentrifuge for 3 min and the RNA-containing supernatant was extracted three times with equal volumes of phenol (Tris-saturated). The RNA was washed

two times with 70% ethanol. RNA was denatured for 5 min at 65° in 50% formamide, 6% formaldehyde in MOPS buffer (20 mM morpholinepropanesulfonic acid, pH 8.0, 5 mM sodium acetate, and 1 mM EDTA). Samples were analyzed by electrophoresis and blotted to nylon membranes. The blots were probed with ^{32}P-labeled nick-translated CHO MT-I and MT-II cDNA probes (31).

Results

The survival curves of CHO-WT, CHO-Cd^r, and CHO-Au^r cells exposed to AF are illustrated in Fig. 2. Both CHO-Au^r (IC_{50} = 11.2 μM) and CHO-Cd^r (IC_{50} = 4.2 μM) cells displayed resistance to the cytotoxic effects of AF as compared to the parent CHO-WT cell line (IC_{50} = 750 nM). The effects of AF treatment on the induction of MT mRNA and MT in these three cell lines were investigated after treatment with 3 μM AF for 3 hr (Fig. 3). AF treatment increased both MT mRNA and MT in the AF-resistant CHO-Cd^r and CHO-Au^r cell lines (Fig. 3A, *lanes 5* and *6*; Fig. 3B, *lanes 4* and *5*). In contrast, AF treatment had no effect on MT mRNA or MT accumulation in the AF-sensitive CHO-WT cell line (Fig. 3A, *lane 4*; Fig. 3B, *lane 3*). Concentrations of 0.25, 0.5, and 1.0 μM AF (data not shown) administered to CHO-WT cells showed no increase in MT mRNA or MT, indicating that the failure of CHO-WT cells to show induction when treated with 3 μM AF was not due to cytotoxicity. Furthermore, $CdCl_2$, a potent inducer of MT in many cell lines, was also unable to cause measurable MT accumulation in the CHO-WT cells (Fig. 3B, *lane 2*), suggesting that the inability of these cells to accumulate MT in the presence of inducer is not specific for AF but is an intrinsic property of these cells.

To determine whether CHO-Cd^r and CHO-Au^r cells display increased resistance to the cytotoxic effects of AF simply because they have the capacity to accumulate MT in the presence of AF, we tested the survival advantage of cells containing high levels of MT prior to AF challenge, reasoning that if MT indeed

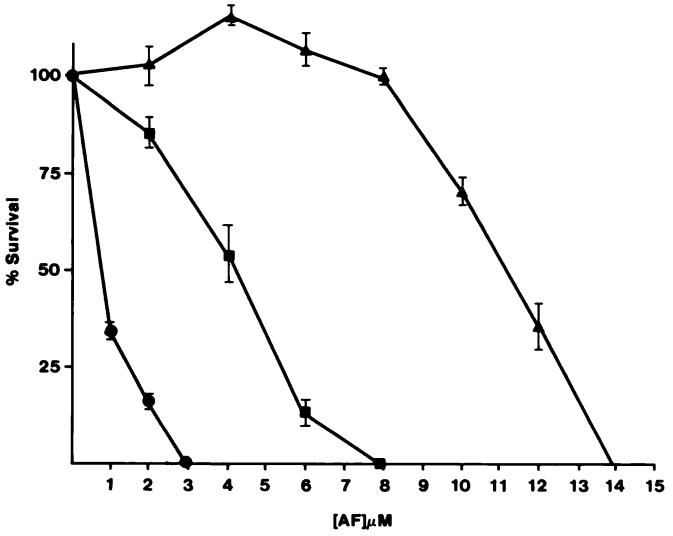

Fig. 2. Survival of CHO-WT, CHO-Cd^r, and CHO-Au$^{r7.5}$ cells during exposure to increasing concentrations of AF. Cells were harvested from monolayers and exposed to different concentrations of AF in suspension at 1 × 10^5 cells/ml for 1 hr. The incubation was carried out at 37° in growth medium in the absence of newborn calf serum. After the incubation, cells were diluted to 1 cell/μl, 5000 cells were plated on Petri dishes and incubated for 4–6 days, and cell survival was assayed by colony-forming ability of the cells (see Materials and Methods). ●, CHO-WT; ■, CHO-Cd^r; ▲, CHO-Au$^{r7.5}$.

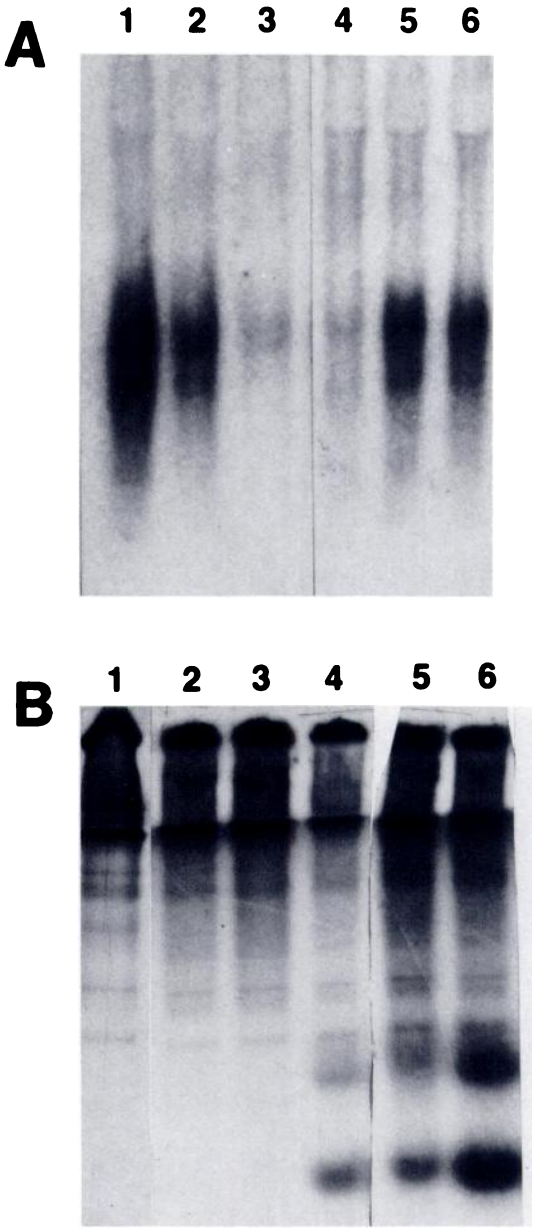

Fig. 3. A. Analysis of MT mRNA induction in CHO-WT, CHO-Cd^r, and CHO-Au$^{r7.5}$ cells in response to AF and $CdCl_2$. Cells were challenged with designated concentrations of the compounds for 12 hr. Total RNA was extracted by the procedure described previously (23). Ten μg of RNA were fractionated by agarose gel electrophoresis in the presence of formaldehyde. The RNA was transferred to nylon membrane (Pall) and hybridized with ^{32}P-nick translated MT-I and MT-II cDNA probes. After hybridization, autoradiographs were developed. *Lane 1*, CHO-Cd^r cells treated with 20 μM cadmium; *lane 2*, CHO-Au^r in the presence of 7.5 μM AF; *lane 3*, CHO-WT with no metal control; *lane 4*, CHO-WT treated with 3 μM AF: *lane 5*, CHO-Cd^r + 3 μM AF; and *lane 6*, CHO-Au^r cells in the presence of 3 μM AF. B. Analysis of MT protein from CHO-WT, CHO-Cd^r, and CHO-Au^r cells by polyacrylamide gel electrophoresis. Cells were challenged with appropriate concentration of cadmium chloride or AF for 12 hr. [^{35}S]Cysteine was added at 4 μCi/ml and incubated further for 8 hr. Cell lysates were prepared as described in Materials and Methods. Aliquots containing equal amounts of radioactivity (50,000 cpm) from each sample were analyzed by polyacrylamide gel electrophoresis. *Lane 1*, CHO-WT cells, untreated control; *lane 2*, CHO-WT cells treated with 5 μM cadmium; *lane 3*, CHO-WT cells treated with 3 μM AF: *lane 4*, CHO-Cd^r cells treated with 3 μM AF; *lane 5*, CHO-Au^r cells grown in the presence of 3 μM AF; and *lane 6*, CHO-Au^r cells treated with 10 μM AF.

confers resistance to AF, cells containing preinduced levels of MT (primed) should display increased resistance to the drug as compared to unprimed cells. $CdCl_2$ was administered to CHO-Cd^r cells for 3 hr before washing and subsequent AF challenge. CHO-Cd^r cells were employed in this study because they are capable of accumulating MT upon $CdCl_2$ challenge and, unlike CHO-Au^r cells, they do not require continuous exposure to AF to maintain resistance to AF (24).[2] Therefore, cells can be maintained in a drug-free environment prior to MT preinduction and MT accumulation during the priming period can be attributed entirely to the priming agent. In addition to simplifying data interpretation, this allowed us to correlate preinduced MT levels with increased resistance to AF.

CHO-Cd^r cells primed with $CdCl_2$ displayed increased resistance to AF-mediated cytotoxicity (Fig. 4). Unprimed cells showed an AF IC_{50} value of 18.5 μM (under these conditions), whereas cells primed with 5 μM and 15 μM $CdCl_2$ showed IC_{50} values of 21.8 μM and 28.5 μM, respectively. Furthermore, the data suggest that increased resistance to AF due to priming with $CdCl_2$ was dose dependent. To explore this observation in more detail, we primed cells with increasing concentrations of $CdCl_2$ for 3 hr, removed the priming agent by washing, challenged the primed cells with an IC_{50} concentration of AF (20 μM under these conditions) for 1 hr, and correlated the sensitivity of these cells with the amount of MT induced at a given concentration of Cd (Fig. 5). Cells primed with 5 μM, 15 μM, and 25 μM $CdCl_2$ were significantly less sensitive to AF-mediated cytotoxicity (Fig. 5B). Maximal resistance was observed in cells primed with 15 μM $CdCl_2$ (>90% survival), and this correlated with the level of resistance observed in cells primed with 15μM $CdCl_2$ and challenged with 20 μM AF in the previous set of priming experiments (Fig. 4) and with the concentration of $CdCl_2$ needed to produce the maximum amount of MT (Fig. 5). However, cells primed with $CdCl_2$ concentrations greater than 15 μM were more sensitive to AF-mediated cytotoxicity than cells primed with 15 μM $CdCl_2$, even though MT levels were maintained maximally at these higher $CdCl_2$ concentrations. In fact, cells primed with 50 μM $CdCl_2$ were 2 times more sensitive than unprimed (control) cells.

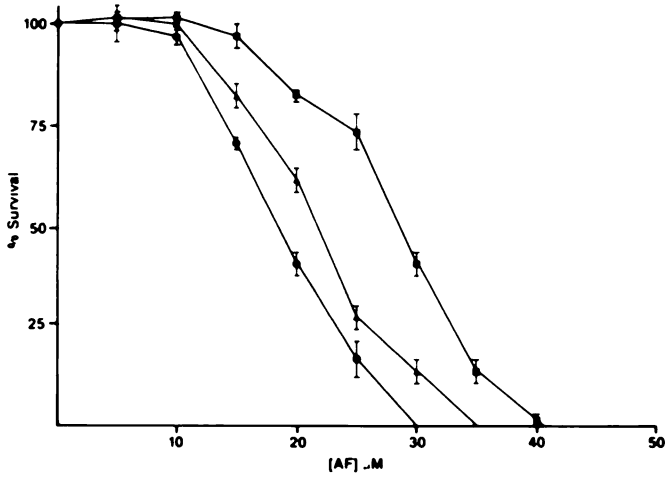

Fig. 4. Effects of pretreatment of CHO-Cd^r cells with Cd on cell survival prior to AF challenge. Five thousand cells were plated on Petri dishes and allowed to attach to the plastic overnight. Cells were untreated (●) or treated with 5 μM (▲) or 15 μM Cd (■) for 3 hr. After 3 hr of cadmium treatment, the monolayers were washed with fresh medium three times. Subsequently, the cells were challenged with increasing concentrations of AF for 1 hr. Fresh medium was added and cell survival was assayed by colony-forming ability 5 days after AF treatment.

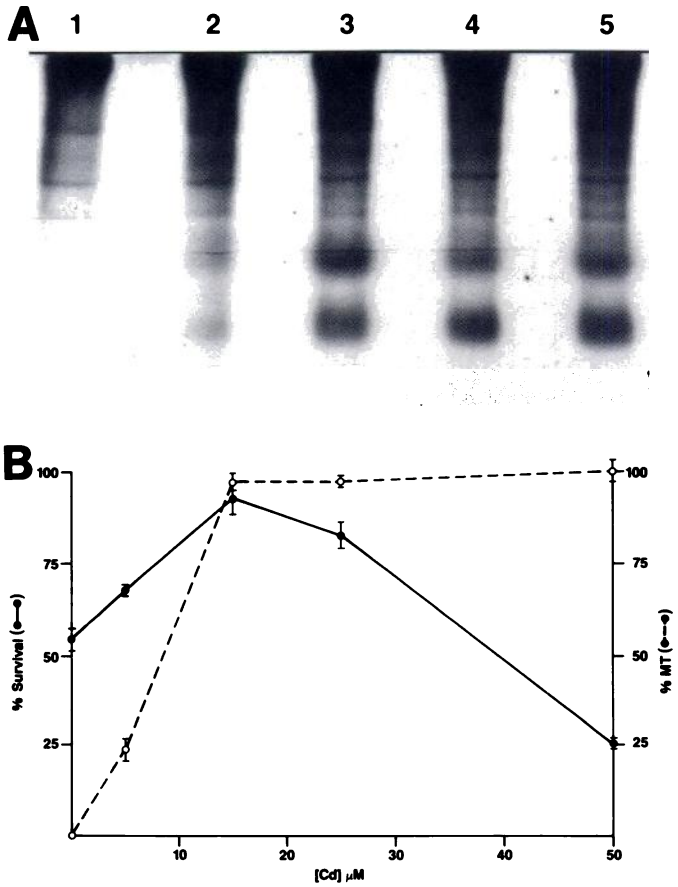

Fig. 5. A. Induction of MT by increasing concentrations of Cd. CHO-Cd^r cells were uniformly labeled with [^{35}S]cysteine for 8 hr and then exposed to indicated concentrations of Cd. Cell lysates were analyzed for MT production by polyacrylamide gel electrophoresis as described in Materials and Methods. *Lane 1*, CHO-Cd^r cells, untreated control; *lane 2*, CHO-Cd^r cells treated with 5 μM $CdCl_2$; *lane 3*, CHO-Cd^r cells treated with 15 μM $CdCl_2$; *lane 4*, CHO-Cd^r cells treated with 25 μM $CdCl_2$; and *lane 5*, CHO-Cd^r cells treated with 50 μM $CdCl_2$. B. Relationship between MT preinduction by Cd and cell survival upon treatment with AF. CHO-Cd^r cells were pretreated with increasing concentrations of $CdCl_2$ for 3 hr as described in Materials and Methods. Immediately after the removal of cadmium, cells were challenged with 20 μM AF (the IC_{50} concentrations of AF under these experimental conditions) for 1 hr. Fresh medium was added and cell survival was assayed by colony-forming ability 5 days after AF treatment. One hundred per cent MT represents the maximal level of MT incuded by Cd as analyzed in Materials and Methods (see autoradiograph in A).

To determine whether the enhanced cytotoxic effects of CHO cells primed with 50 μM $CdCl_2$ and then challenged with AF were due to an intrinsic cytotoxic effect of $CdCl_2$, a cell survival assay was performed in which CHO-Cd^r cells were challenged with $CdCl_2$ under the conditions employed in the priming experiments (Fig. 6). Results from these experiments showed that cell death was not induced until concentrations of $CdCl_2$ exceeding 250 μM were applied. Thus, it is unlikely that the enhanced cytotoxic effects observed in CHO-Cd^r cells primed with 50 μM $CdCl_2$ are due to cytotoxic levels of $CdCl_2$.

[2] B. P. Monia, T. R. Butt, C. K. Mirabelli, D. J. Eker, E. Sternberg, and S. T. Crooke, unpublished observations.

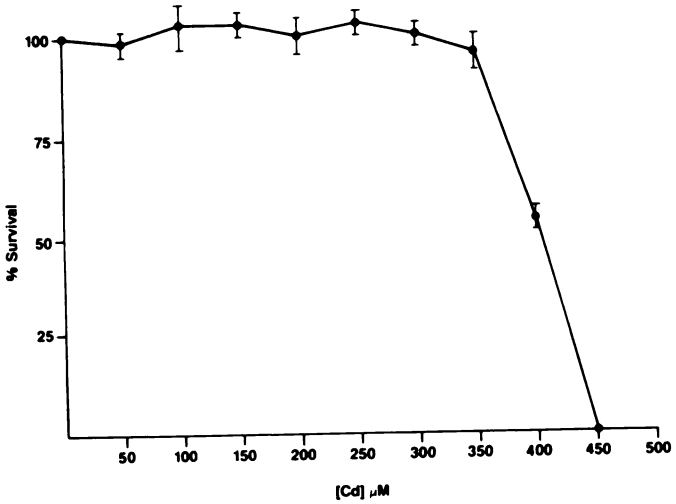

Fig. 6. Cell survival assay of CHO-Cd^r cells exposed to Cd. Five thousand CHO-Cd^r cells were plated on 60 × 15 mm Petri dishes and allowed to adhere to plastic overnight. Cells were then treated with increasing concentrations of $CdCl_2$ for 3 hr and washed thoroughly. Cell survival was determined by colony-forming ability as described previously.

Discussion

Increased resistance to AF in CHO-Cd^r cells is strongly associated with induction of MT and, as indicated by our studies, increased cellular accumulation of MT appears to play a major role in the mechanisms underlying cellular resistance to AF. This conclusion is based on the following observations.

Induction of MT mRNA and MT in the presence of both AF and Cd was rapid and substantial in the AF-resistant CHO-Au^r cells, whereas no induction was observed in the highly sensitive CHO-WT cells. Furthermore, induction of MT by AF was also observed in the partially resistant CHO-Cd^r cells. The mechanism by which CHO-Au^r cells exhibit a greater level of resistance to AF than the CHO-Cd^r cells is unclear (23, 28). It is possible that the growth of cells in the presence of AF for extended periods of time can enhance the rate of MT gene transcription or cause the induction of proteins other than MT which can provide an additional survival advantage to cells grown in the presence of AF. Since the rate of Au-MT degradation is constant in these cells, we conclude that resistance to gold is due to increased rate of MT production (24). Regardless of the mechanisms of increased resistance in CHO-Au^r, these findings suggest that the capacity of the cell to accumulate MT when challenged with AF is a prerequisite for the resistance to the drug.

Preinduction of low levels of MT was observed with 5 μM Cd which conferred a small, but significant, increase in AF resistance, whereas 15 μM Cd induced maximal MT levels and conferred maximal resistance to AF. Induction of CHO-Cd^r cells with concentrations ≤25 μM Cd caused additional resistance to AF. The effect of Cd correlated with the concentration-dependent increase in MT mRNA and MT protein levels. At concentrations greater than 15 μM Cd, no additional increases in MT mRNA or MT protein were observed. Moreover, at concentrations greater than 25 μM Cd, AF toxicity was enhanced. Thus, we conclude that preinduction of MT by Cd results in enhanced protection from AF toxicity because larger amounts of MT are induced by Cd than are normally required to protect from Cd toxicity. Although we have not directly measured the degree of Cd saturation in MT molecules, it is likely that the MT molecules preinduced by 15 μM Cd are not fully saturated with Cd. The unsaturated MT thus serves as a sulfhydryl-rich "sink" to which intracellular gold can bind, rendering it less toxic to cellular targets. Furthermore, we can conclude that in these cells maximal induction of MT by Cd occurs at 15–25 μM, and this is substantially less than the concentration at which the toxicity is observed. Thus, Cd toxicity in these cells is due to an array of nonspecific interactions with a variety of targets thus requiring relatively high intracellular concentrations of Cd.

In conclusion, the present study provides additional evidence that MT mediates a significant proportion of the resistance to AF. It demonstrates that, at 15–25 μM Cd concentration, the MT promoter is maximally active in these cells, and it clearly shows that Cd priming protects from AF toxicity by induction of MT. Perhaps even more importantly, this study provides experimental data to support the notion that gold complexes may be selectively cytotoxic to a phenotypically defined subpopulation of cells (32) and that the degree of sensitivity of a subpopulation of cells is directly related to their ability to accumulate intracellular levels of MT. Finally, it suggests that the mechanism of Cd toxicity may be nonspecific or that these cells possess mechanisms of resistance to Cd which are different from induction of MT.

Many metals are capable of inducing MT mRNA but only a subset of these appears to be detoxified by MT (14). Furthermore, not all metals or metal-containing drugs can induce MT accumulation (23, 24, 27, 29, 31, 33). It has been reported that MT can bind Au *in vitro* (19–22) and that Au can induce MT gene transcription (23). These earlier reports implicated MT as a potential mechanism of Au detoxification in cells. The results of the present investigation suggest that MT is capable of detoxifying the gold-containing compound AF, and implicate it in cellular resistance to gold compounds in general. Further investigations may clarify the question of whether the mechanism of resistance demonstrated here is applicable to the development of insensitivity or tolerance to AF and other gold compounds in the clinic (32).

References

1. Sutton, B. M., E. McGusty, D. T. Wab, and M. T. DiMartino. Oral gold. Antiarthritic properties of alkylphosphinegold coordination complexes. *J. Med. Chem.* **15**:1095–1098 (1972).
2. Heuer, M. A., and R. W. Morris. Smith Kline and French worldwide clinical experience with auranofin: a review, in *Auranofin* (Current Clinical Practice Series 7) (H. A. Capell, D. S. Cole, M. K. Manghani, and R. W. Morris, eds.). Excerpta Medica, Amsterdam, 474–503 (1983).
3. Sutton, B. M. In *Platinum, Gold and Other Metal Chemotherapeutic Agents* (S. J. Lippard, ed.). American Chemical Society, Washington, D. C., 356–369 (1983).
4. Lee, J., L. Rebar, S. DeMuth, and N. Hanna. Suppressed IL-2 production in adjuvant arthritic rats: role of suppressor cells and effect of auranofin treatment. *J. Rheumatol.* **12**:885–891 (1985).
5. Snyder, R. M., C. K. Mirabelli, and S. T. Crooke. Cellular association, intracellular distribution and efflux of auranofin via sequential ligand exchange reactions. *Biochem. Pharmacol.* **35**:923–932 (1986).
6. Allaudeen, H. S., R. M. Snyder, M. H. Whitman, and S. T. Crooke. Effect of coordinated gold compounds on *in vitro* and *in situ* DNA replication. *Biochem. Pharmacol.* **34**:3243–3250 (1985).
7. Simon, T. M., D. H. Kunishima, G. J. Vibert, and A. Lorber. Screening trial with the coordinated gold compound auranofin using lymphocytic leukemia P388. *Cancer Res.* **41**:94–97 (1981).
8. Mirabelli, C. K., R. K. Johnson, C. M. Sung, L. Faucette, K. Muirhead, and S. T. Crooke. Evaluation of the *in vivo* antitumor activity and *in vitro* cytotoxic properties of auranofin, a coordinated gold compound, in murine tumor models. *Cancer Res.* **45**:32–39 (1985).
9. Mirabelli, C. K., R. K. Johnson, D. T. Hill, L. Faucette, G. Girard, G. Kuo, C. M. Sung, and S. T. Crooke. Correlation of *in vitro* cytotoxic and *in vivo*

antitumor activities of gold(I) coordination complexes. *J. Med. Chem.* **29**:218–223 (1986).
10. Foulkes, E. C. *Biological Roles of Metallothionein.* Elsevier, New York (1982).
11. Butt, T. R., E. Sternberg, J. Herd, and S. T. Crooke. Cloning and expression of a yeast copper metallothionein gene. *Gene (Amst.)* **27**:23–33 (1984).
12. Butt, T. R., E. J. Sternberg, J. A. Gormon, P. Clark, D. Hammer, M. Rosenberg, and S. T. Crooke. Copper metallothionein of yeast, structure of the gene and regulation of expression. *Proc. Natl. Acad. Sci. USA* **81**:3332–3336 (1984).
13. Karin, M., and R. Richards. Human metallothionein genes—primary structure of metallothione II gene and a related processed gene. *Nature (Lond.)* **299**:797–802 (1982).
14. Durnam, D. M., and R. D. Palmiter. Induction of metallothionein-I mRNA in cultured cells by heavy metals and iodoacetate: evidence for gratuitious inducer. *Mol. Cell. Biol.* **4**:484–491 (1984).
15. Karin, M., R. D. Anderson, E. Slater, K. Smith, and H. R. Herchman. Metallothionein mRNA induction in HeLa cells in response to zinc or dexamethasone in a primary induction response. *Nature (Lond.)* **286**:295–297 (1980).
16. Karin, M., A. Heslinger, H. Hotgreve, R. I. Richard, P. Krauker, H. M. Westphal, and M. Beata. Characterization of DNA sequences through which cadmium and glucocorticoid hormones induce human metallothionein-II gene. *Nature (Lond.)* **308**:513–519 (1984).
17. Friedman, R. L., and G. R. Stark. Interferon-induced transcription of HLA and metallothionein genes containing homologous upstream sequences. *Nature (Lond.)* **314**:637–639 (1985).
18. Carter, A. D., B. K. Felber, M. Walling, M. Jubier, C. J. Schmidt, and D. H. Hamer. Duplicated heavy metal control sequences of the mouse metallothione I gene. *Proc. Natl. Acad. Sci. USA* **81**:7392–7396 (1984).
19. Schmitz, G., D. T. Minkel, D. Gingrich, and C. F. Shaw III. The binding of gold I to metallothionein. *J. Inorg. Biochem.* **12**:293–306 (1980).
20. Sharma, R. P., and E. G. McQueen. The effect of zinc and copper pretreatment on the binding of gold I to hepatic and renal metallothioneins. *Biochem. Pharmacol.* **31**:2153–2159 (1982).
21. Mogilnicka, E. M., and J. K. Piotrowski. Inducible gold binding proteins in rat kidneys. *Biochem. Pharmacol.* **31**:2153–2159 (1982).
22. Glennas, A., P. E. Hunziker, J. S. Garvey, J. H. R. Kagi, and H. E. Rugstad. Metallothionein in cultured human epithelial cells and synovial rheumatoid fibroblasts after *in vitro* treatment with auranofin. *Biochem. Pharmacol.* **35**:2033–2040 (1986).
23. Butt, T. R., E. J. Sternberg, C. K. Mirabelli, and S. T. Crooke. Regulation of metallothionein gene expression in mammalian cells by gold compounds. *Mol. Pharmacol.* **29**:204–210 (1986).
24. Monia, B. P., T. R. Butt, D. J. Ecker, C. K. Mirabelli, and S. T. Crooke. The rate of metallothionein turnover in mammalian cells is affected by the metals it binds. *J. Biol. Chem.* **261**:10957–10959 (1986).
25. Dinn, W., and J. Frazier. Protective effect of metallothionein on cadmium toxicity in isolated rat hepatocytes. *Biochem. J.* **230**:395–402 (1985).
26. Nielson, K. B., C. L. Ackin, and D. R. Winge. Distinct metal-binding configurations in metallothionein. *J. Biol. Chem.* **260**:5342–5350 (1985).
27. Hildebrand, C. E., R. A. Tobey, E. W. Campbell, and M. D. Enger. A cadmium resistant variant of the Chinese hamster cell with increase metallothionein induction capacity. *Exp. Cell Res.* **124**:237–246 (1979).
28. Crawford, B. D., M. D. Enger, B. B. Griffith, J. K. Griffith, J. L. Hanners, J. L. Longmire, A. C. Munk, R. L. Stallings, J. G. Termer, R. A. Walters, and C. E. Hildebrand. Coordinate amplification of metallothionein I and II genes in cadmium-resistant Chinese hamster cells: implications for mechanisms regulating metallothionein gene expression. *Mol. Cell. Biol.* **5**:320–329 (1985).
29. Thomas, P. S. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. *Proc. Natl. Acad. Sci. USA* **77**:5201–5205 (1980).
30. Bresser, J., H. Hubbell, and D. Gillespie. Biological activity of mRNA immobilized on nitrocellulose in Na I. *Proc. Natl. Acad. Sci. USA* **80**:6523–6527 (1985).
31. Griffith, B. B., R. A. Walters, M. D. Enger, C. E. Hildebrand, and J. K. Griffith. DNA cloning and nucleotide sequence comparison of Chinese hamster metallothionein I and II mRNA. *Nucleic Acid Res.* **11**:901–910 (1983).
32. Crooke, S. T., and C. K. Mirabelli. Molecular mechanisms of action of auranofin and other gold complexes as selected to their biologic activities. *Am. J. Med.* **10**:109–113 (1983).
33. Prestayko, A. W., S. T. Crooke, and S. K. Carter. *Cis Platin: Current Status and New Developments.* Academic Press, New York (1980).

Send reprint requests to: Dr. Stanley T. Crooke, Smith Kline and French Laboratories, Department of Molecular Pharmacology, P. O. Box 7929, 1500 Spring Garden Street, Philadelphia, PA 19101.