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Messenger RNA Loses the Ability to Direct *In Vitro* Peptide Synthesis following Incubation with Cisplatin

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SUMMARY

Inhibition of *in vitro* translation activity by the chemotherapeutic agent cisplatin has been studied. Peptide synthesis was measured in translation assays prepared from guinea pig and rabbit reticulocyte lysates. There is a concentration- and time-dependent inactivation of translation by cisplatin. Inhibition is nearly maximal after a 30-min exposure. Translation is inhibited by cisplatin at concentrations that have been found after the administration of therapeutic doses. Evidence suggests that the suppression of peptide synthesis is due to an interaction between cisplatin and mRNA. An apparent qualitative difference in the peptide products translated from mRNA exposed to cisplatin is demonstrated. Large peptide products are inhibited to a greater extent than products with faster electrophoretic mobility. Decreased translation activity cannot be explained by digestion of the message by either cisplatin itself or by some contaminant of the drug solution.

Cisplatin, *cis*-diamminedichloroplatinum (II), is a chemotherapeutic agent that has demonstrated antitumor activity against a number of human neoplasms (1). Several mechanisms of cytotoxicity have been proposed for cisplatin. Among these, binding of the drug to DNA has been well documented (2–4). Similar binding to RNAs, although not explored as thoroughly, has been suggested (5). If cisplatin binds RNA, then an effect on the synthesis of polypeptides might be anticipated.

Previous studies examining the relationship between cisplatin and protein synthesis have produced variable results (6–8). These studies were carried out in whole animal or cell culture preparations. Such *in vivo* analyses of the translational process are confounded by the interdependence upon related cellular activities such as DNA replication and transcription.

It is possible to study translation and measure *in vitro* peptide synthesis by the use of reticulocyte lysates. These preparations contain the enzymes and related components necessary to synthesize peptides in the absence of other cellular processes. The purpose of this study is to examine the effect of cisplatin on the translation of peptides in a cell-free system.

Materials and Methods

Reticulocytes were obtained from male English short-hair guinea pigs [300–500 g; strain, Mdh:(SR[A])] from the Michigan State Health Laboratories, Lansing, MI) made anemic by subcutaneous injections of 1.7% phenylhydrazine at a dose of 17 mg/kg on 4 consecutive days. The guinea pigs were allowed to recover for 4 days, then bled on the eighth day (9). Reticulocytes were isolated and lysed by osmotic shock as described for rabbit reticulocyte lysate (10). Rabbit reticulocyte lysates were obtained from Green Hecters (Oregon, WI). All rabbit reticulocyte lysates were prepared for translation essentially as described (10). The modifications involved in preparing guinea pig lysates for translation included adding hemin to 0.1 mM and creatinine phosphokinase to 3.5 units/ml of lysate, with no addition of dithiothreitol (9). For translation of exogenous BMV mRNA (Promega Biotec, Madison, WI), the endogenous mRNA in the lysate was digested with a calcium-dependent micrococcal nuclease (Pharmacia Biotechnology, Molecular Biology Division, Piscataway, NJ) (9, 10). Lysate prepared for translating endogenous mRNA includes water in place of the nuclease and EGTA. Translation was measured as the incorporation of [^{35}S]methionine (>800 Ci/mmol; Amersham Corporation, Arlington Heights, IL), [^{35}S]cysteine (>600 Ci/mmol; Amersham Corporation), or L-[3,4,5-^{3}H(N)]leucine (>110 Ci/mmol; New England Nuclear Research Products, Boston, MA). To start the translation assay, a reaction cocktail was added containing 2.7 mM spermidine (free base; Sigma Chemical Company, St. Louis, MO), 0.7 mM GTP (sodium salt; Sigma Chemical Company), and a labeled amino acid (final concentration in the translation assay of 0.5 μCi/ml). The reaction cocktail also contains biosynthesis reaction mixture (Bethesda Research Laboratories, Gaithersburg, MD), which was ⅔ of the total reaction cocktail volume. A typical assay consisted of 16 μl of reticulocyte lysate, a 4 μl addition, and 5 μl of reaction cocktail. The 4 μl addition consisted of cisplatin and/or dithiobiuret in the appropriate translation assays. In translation assays directed by exogenous mRNA, 2 μl of the 4 μl contained 1.0 μg of BMV mRNA. Cisplatin (pure compound; Bristol-Myers Company, Syracuse, NY) and dithiobiuret (American Cyanamid Company, New York, NY) were dissolved in diethylpyrocarbonate-treated (10) deionized water immediately before use. The 4 μl was added to lysate and allowed to preincubate according to the experimental protocol. The reaction cocktail was then combined with this solution and the trans-

ABBREVIATIONS: BMV, brome mosaic virus; TCA, trichloroacetic acid; SDS, sodium dodecyl sulfate; EGTA, ethyleneglycol-bis-(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid.

lation reaction was initiated. Translation was allowed to proceed for 60 min at 37°. After this incubation, 10 μl of the translation assay was hydrolyzed in 1 ml of 1 N sodium hydroxide at 37° for 10 min [assays incorporating [^{35}S]cysteine were alkylated before the hydrolysis (11)]. The translation products were then filtered after precipitation in 25% cold TCA containing 2% w/v casein, acid hydrolysate (Sigma Chemical Company). The filters were dissolved in 0.5 ml of tissue solubilizer (TS-1; Research Products International Corporation, Mount Prospect, IL) for 30 min at 60°. Once cooled, scintillation cocktail (10 ml of aqueous counting scintillant; Amersham Corporation) was added and the samples were counted by liquid scintillation spectrometry. Translation activity is expressed as counts incorporated into TCA-precipitable material minus the unincorporated background counts. RNasin was purchased from Promega Biotec.

Results

The addition of cisplatin results in a large decrease in the ability of reticulocyte lysates to incorporate radiolabeled amino acids into TCA-precipitable material. This decrease reflects an inhibition of peptide synthesis.

To characterize the effect of cisplatin on peptide synthesis the time course of the inhibition by cisplatin was examined. This effect on translation was observed in *in vitro* preparations from two species, rabbit and guinea pig (Fig. 1). Lysates were preincubated with concentrations of cisplatin for various periods of time up to 60 min. As shown, cisplatin inhibition increases during the time of preincubation with the lysate. The figure also demonstrates that larger concentrations of the drug result in increased inhibition. Lysates from the two species behave similarly, both in terms of the effective concentrations and the time course of the effect.

The relationship between cisplatin concentration and inhibition of peptide synthesis was more thoroughly examined. As shown in Fig. 1, the cisplatin effects on translation are near maximal after 30 min, therefore, this length of time was chosen for the preincubation in subsequent experiments. Concentrations of cisplatin between 3 and 800 μM were incubated with guinea pig and rabbit reticulocyte lysates. These were then assayed for their ability to incorporate amino acids into TCA-precipitable material. The concentration dependence of the cisplatin inhibition of translation of endogenous mRNA into peptides is shown in Fig. 2. In the guinea pig system, cisplatin detectably inhibits peptide synthesis at a concentration as low as 3 μM. A concentration of 39 μM gives 50% inhibition. Synthesis with rabbit lysate is also detectably inhibited at the lowest concentration of cisplatin tested (4 μM). The IC_{50} for cisplatin inhibition of translation of endogenous mRNA is 98 μM in rabbit reticulocyte lysates. Best fit lines are plotted for both species. The guinea pig and rabbit best fit lines are parallel, having slopes in their linear regions of 48 and 47% inhibition/log [cisplatin], respectively.

Cisplatin inhibition of the incorporation of labeled amino acid was also demonstrated in translation assays directed by exogenously supplied mRNA. The endogenous mRNA in reticulocyte lysates was digested with nuclease. Translation was then carried out by adding BMV mRNA. Peptide synthesis is inhibited with an IC_{50} of 59 μM cisplatin in these translation assays. This IC_{50} is similar to those from rabbit and guinea pig assays of endogenous mRNA.

By using nuclease-treated exogenous mRNA-dependent lysates, it was possible to test whether cisplatin inhibits peptide synthesis by an interaction with mRNA or with other components of the translation system. Various concentrations of cisplatin were preincubated with either mRNA, the mRNA-dependent lysate, or both fractions (Fig. 3). Only when cisplatin was preincubated with the mRNA was the full inhibitory effect observed. When the mRNA fraction was incubated with 30 μM cisplatin then added to a nuclease-treated lysate, peptide synthesis was inhibited 85%. In contrast, when a nuclease-treated lysate was incubated with the same concentration of cisplatin, then mRNA was added, translation was inhibited only 19%. The fact that inhibition is much greater when the drug is preincubated with the exogenous mRNA than it is when lysate without message is preincubated with the drug suggests that an

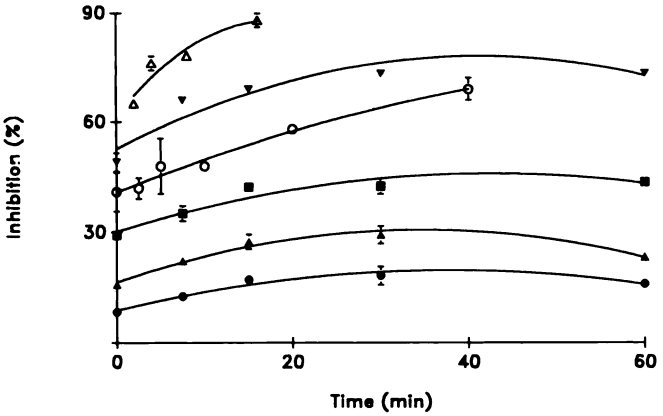

Fig. 1. Time course of cisplatin inhibition of *in vitro* translation. Rabbit reticulocyte lysates were preincubated with 30 (●), 60 (▲), 96 (■), and 240 (▼) μM cisplatin for 0, 7.5, 15, 30, or 60 min at 22°. Guinea pig reticulocyte lysates were preincubated at 30 μM cisplatin (○) for 0, 2.5, 5, 10, 20, and 40 min and at 100 μM cisplatin (△) for 2.5, 5, 10, and 20 min at 22°. Lysate was prepared for the translation of endogenous mRNA as described in Materials and Methods. In order to examine the progression of cisplatin inhibition at several time points yet minimize the loss of translation activity that occurs at room temperature, lysate was aliquoted into assay tubes that were placed on dry ice. At the appropriate time before the start of translation, assay tubes were thawed and combined with cisplatin. Inhibition of translation was determined by comparison with assays preincubated for identical times in the absence of cisplatin. Each *point* represents six individual assays. *Error bars* represent ± 1 SE. Best fit lines were plotted by second order regression.

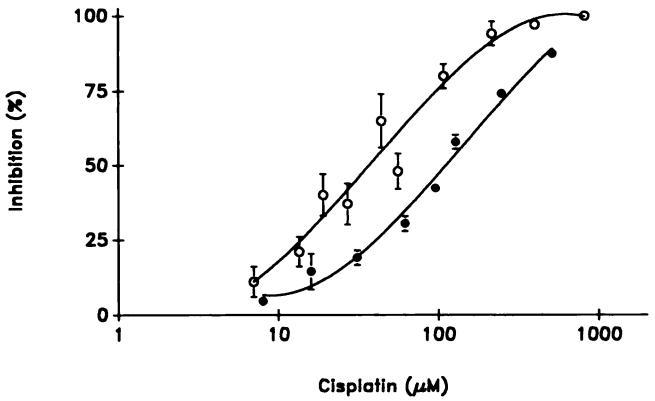

Fig. 2. Relation of cisplatin concentration to the extent of inhibition of translation activity in reticulocyte lysates. Endogenous mRNA was translated by guinea pig (○) and rabbit (●) lysates after preincubation with increasing concentrations of cisplatin for 30 min at 22°. Inhibition of translation was determined by comparison with assays preincubated in the absence of cisplatin. Each *point* represents six individual assays. *Error bars* represent ± 1 SE. Best fit lines were plotted by third order regression.

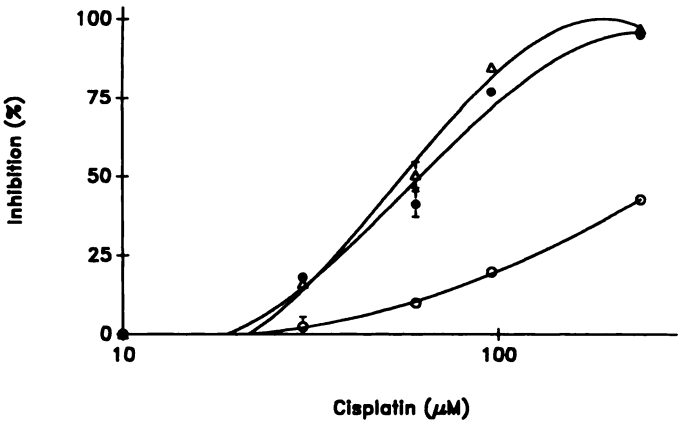

Fig. 3. Inhibition of translation by cisplatin follows preincubation with exogenous mRNA in a message-dependent translation assay. Cisplatin at concentrations of 0, 30, 60, 96, or 240 μM was preincubated with either rabbit mRNA-dependent lysate (○), BMV mRNA (●), or both fractions (△) for 30 min at 22°. After the preincubation, the mRNA and lysate were combined and translation was measured. Inhibition of translation was determined by comparison with the assays preincubated in the absence of cisplatin. Each *point* represents six individual assays. *Error bars* represent ± 1 SE. Best fit lines were plotted by third order regression.

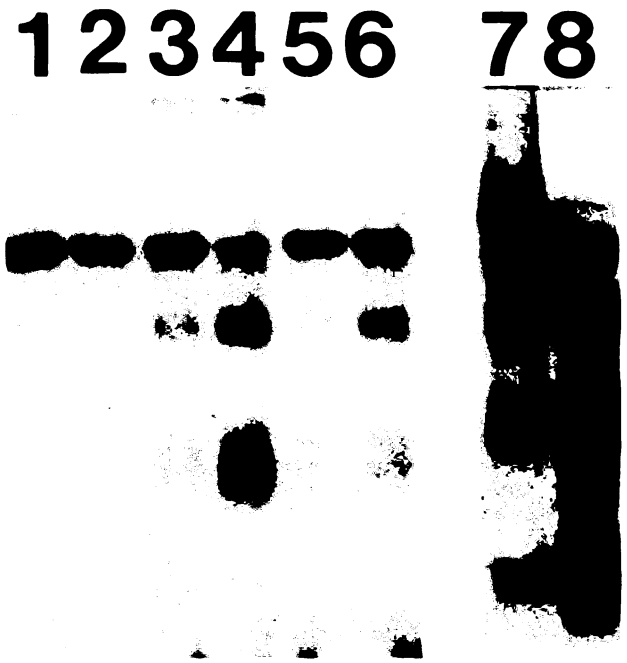

Fig. 4. Qualitative differences in translation products from cisplatin-exposed assays. Products from cisplatin-inhibited translation assays were electrophoretically separated on 7.5% SDS-polyacrylamide gels (18). The gels were fixed in 10% acetic acid and fluorographed as described (19). The lanes were loaded as described in the text. *Lanes 1–6* were each loaded with an equal number of dpm. These show the products of translation directed by endogenous rabbit mRNA. *Lane 1* was preincubated 30 min at 22° in the absence of cisplatin while *lanes 2, 3,* and *4* were preincubated with 16, 96 and 512 μM cisplatin, respectively. Product in *lanes 5* and *6* were each preincubated with 100 μM cisplatin for 2.5 and 40 min, respectively. *Lanes 7* and *8*, each loaded with an equal number of dpm, show products from the translation of BMV mRNA by message-dependent rabbit lysate. Both assays were preincubated at 22° for 30 min, *lane 7* in the absence and *lane 8* in the presence of 96 μM cisplatin.

interaction between cisplatin and the mRNA is critical for the reduction in peptide synthesis activity.

Although the inhibition of translation by cisplatin can be explained by an interaction with the mRNA, it was still possible that inhibition was also dependent upon the particular label used to measure peptide synthesis. Therefore, three different amino acids, [^{3}H]leucine, [^{35}S]cysteine, and [^{35}S]methionine, were tested. Essentially the same inhibition of translation by cisplatin was found with each of the radiolabeled amino acids. Translation, in lysates containing endogenous mRNA preincubated with 114 μM cisplatin, was measured as the incorporation of [^{3}H]leucine, [^{35}S]cysteine, or [^{35}S]methionine into TCA precipitates. In these assays, almost the same inhibition of translation was found, 73, 76, and 72%, respectively.

To determine whether cisplatin caused qualitative changes in the peptide products being synthesized, they were analyzed by SDS-polyacrylamide gel electrophoresis (Fig. 4). Samples in lanes *1–6* were withdrawn from rabbit endogenous mRNA assays exposed to various concentrations of the drug for different lengths of time. Larger volumes of the inhibited translation assays were electrophoresed so that comparable amounts of incorporated radioactivity were placed in each lane of the gel. The total TCA-precipitable radioactivity loaded into each of the lanes *1–6* was 5200 dpm. The assay in lane *1* was not exposed to cisplatin. The lanes *2–4* are analyses of translation assays incubated with 16, 96, and 512 μM cisplatin, which were inhibited 9, 45, and 87%, respectively. Lanes *5* and *6* show the translation products of lysates preincubated with 100 μM cisplatin for 2.5 and 40 min, which were inhibited 45 and 71%, respectively. The intensity of the bands, or amount of radioactivity associated with each polypeptide, decreases more for the larger, more slowly migrating bands, than for the rapidly migrating peptides. Because equal counts were loaded into each lane, the differences in the banding of the more inhibited lanes are emphasized. Loading the same number of counts of samples containing less large product means that more label is carried in the form of smaller peptides. Products of a mRNA-dependent translation assay directed by BMV mRNA also demonstrate a more profound decrease in high molecular weight products. Equal amounts of incorporated amino acid (35,671 dpm in each lane) were also compared in lanes *7* and *8*. Lane *7* contains product from an assay not exposed to cisplatin. The assay in lane *8* was preincubated with 96 μM cisplatin, which resulted in 85% inhibition.

The extent of inhibition of the various peptide products was quantitated. Another gel was prepared analyzing equal volumes from assays translating BMV mRNA. Two lanes were loaded with product, one from an assay without drug and in the other the mRNA was preincubated with 96 μM cisplatin, which resulted in 85% inhibition. These lanes were each cut from the gel and sliced into fractions of equal length so that the counts per minute in fractions with the same migration could be compared. For each fraction from the cisplatin-exposed assay, the amount of inhibition was calculated relative to the uninhibited assay (Fig. 5). Large peptide products are inhibited nearly 90% whereas smaller peptides are inhibited on the order of 40% when compared with assays run in the absence of cisplatin.

Experiments were then conducted to reverse the effects of cisplatin on translation. Substances that interact with this drug should alter its ability to inhibit peptide synthesis. A reaction of cisplatin with the chelator dithiobiuret can be demonstrated spectrophotometrically (Fig. 6). Neither cisplatin nor dithiobiuret show any increase in absorbance over time at 310 nm.

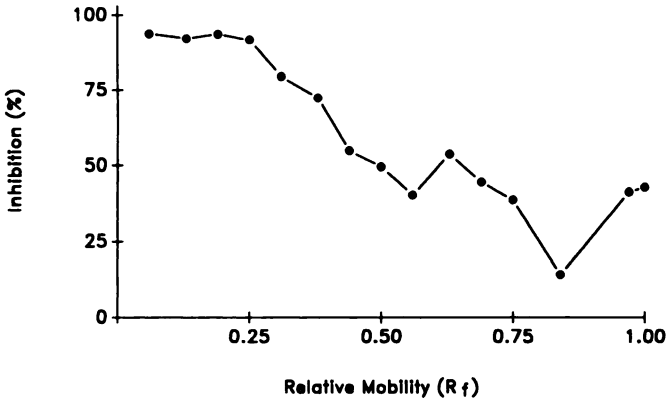

Fig. 5. Cisplatin preferentially inhibits translation products with slower electrophoretic mobility. An equal volume of products from two translation assays were electrophoretically separated on a 7.5% SDS-polyacrylamide gel (18). The gel was fixed in 10% acetic acid and fluorographed as described (19). The assays were preincubated 30 min at 22°, one with 96 μM cisplatin and the other in the absence of drug. The lanes were cut from the gel and then into 0.7 × 1.0 cm fractions. The fractions were rehydrated in 0.15 ml of water for 1 hr at 22° and dissolved by incubation with 0.5 ml of hydrogen peroxide at 80° for 4 hr. After the vials cooled, 10 ml of scintillation cocktail were added to each sample and they were counted by liquid scintillation. Inhibition of translation of the separated products was determined by comparing the counts in segments representing equal migration from the cisplatin-pretreated and untreated translation assays.

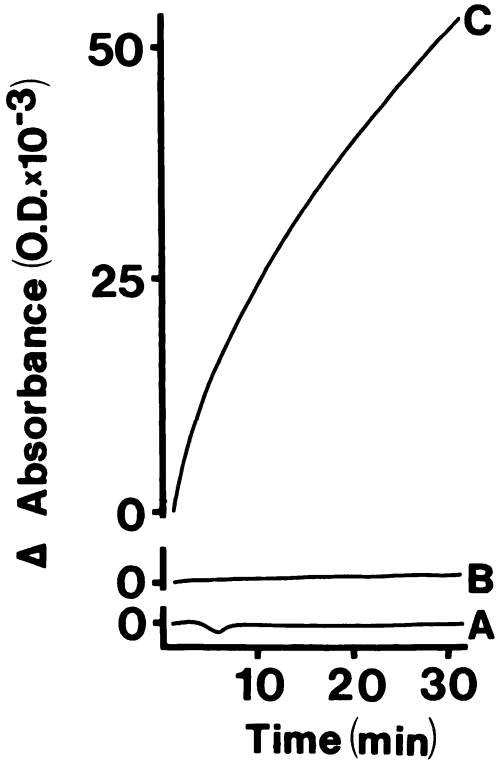

Fig. 6. Reaction of cisplatin with dithiobiuret. The change in absorbance was recorded at 310 nm as a function of time using a Beckmann UV 5260 recording spectrophotometer. Changes in absorbance with time are shown for solutions of 3.3 mM dithiobiuret (*line A*) and 1.0 mM cisplatin (*line B*). Equal volumes of the dithiobiuret and cisplatin solutions were then combined (*line C*) and the change in absorbance was measured. Both compounds were prepared in 0.1 M sodium acetate buffer, pH 5.0. The reference cell contained the same buffer.

When cisplatin is combined with dithiobiuret, however, absorbance increases over time. This increase in absorbance suggests that a reaction occurs between the two compounds.

The possibility that dithiobiuret could reverse the cisplatin inhibition of translation was then examined. Addition of dithiobiuret to reticulocyte lysates has neither an inhibitory nor a stimulatory effect on peptide synthesis. When dithiobiuret and cisplatin are combined before addition to a translation assay, the inhibitory action of cisplatin on peptide synthesis is reduced (Fig. 7). Higher concentrations of this chelator more greatly reverse the cisplatin effect. If lysate and dithiobiuret are combined before addition of cisplatin or if lysate and cisplatin are combined before dithiobiuret, then reversal of the cisplatin effect does not occur and translation activity is inhibited. In the mRNA-dependent assay, the addition of precombined cisplatin and dithiobiuret to mRNA causes a reversal of the cisplatin effect. However, if cisplatin and mRNA are combined before addition of dithiobiuret, reversal does not occur. Other metal chelators tested, EDTA and EGTA, did not antagonize cisplatin. Aluminum is reported to react with and inactivate cisplatin (12) but it also lacked the ability to reverse the inhibition.

The possibility that the cisplatin acts to degrade mRNA or that the drug was contaminated by a ribonuclease was ruled out by analyzing a sample of mRNA, preincubated with cisplatin, by agarose gel electrophoresis. This sample was compared with an identical sample of mRNA not preincubated with the drug (Fig. 8). The resulting electrophoretic patterns are not distinguishable. The apparent lack of degradation of the mRNA incubated with the drug indicates that the cisplatin solution does not have a ribonuclease activity. Furthermore, the cisplatin inhibition of translation is not reversed by the ribonuclease inhibitor RNasin.

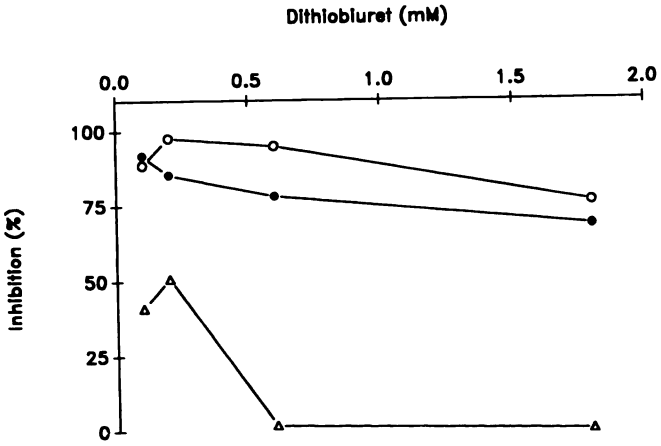

Fig. 7. Dithiobiuret reverses the inhibition of translation by cisplatin. Dithiobiuret at 0.1, 0.2, 0.6, and 1.8 mM concentration was preincubated with 200 μM cisplatin (△) for 7 min at 22°. In another set of assays, rabbit lysate containing endogenous mRNA (●) was preincubated with the same concentrations of dithiobiuret for 7 min at 22°. The dithiobiuret-cisplatin solution was then added to lysate containing endogenous mRNA and further preincubated 30 min at 22°. The dithiobiuret-lysate mixture was further preincubated with 200 μM cisplatin for 30 min at 22°. In a third set of assays, lysate containing endogenous mRNA was preincubated with 200 μM cisplatin for 7 min at 22° followed by another 30 min preincubation with either 0.1, 0.2, 0.6, or 1.8 mM dithiobiuret (○). The inhibition was calculated for each set of experiments by comparison with a similarly combined preincubated assay with water substituted for either the dithiobiuret or cisplatin.

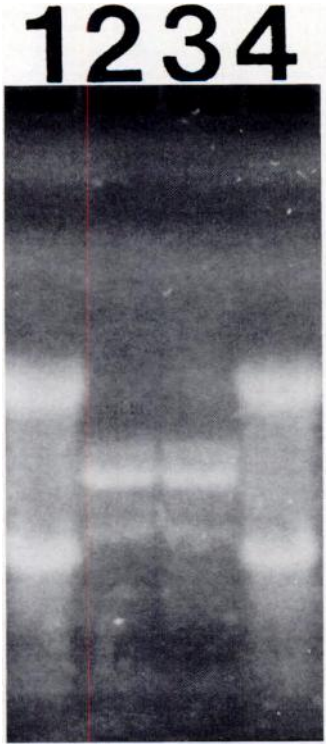

Fig. 8. Messenger RNA is not degraded by incubation with a cisplatin solution. BMV mRNA (3.5 μg), after an incubation in the absence (*lane 2*) or presence (*lane 3*) of 300 μM cisplatin for 30 min at 22°, was analyzed by electrophoresis in a 0.8% agarose gel. RNAs were denatured with glyoxal and dimethyl sulfoxide before electrophoresis and were stained with ethidium bromide as described (10). Glyoxylated total RNA was loaded in *lanes 1* and *4*; the 18 S and 28 S ribosomal bands are visible.

Discussion

Cisplatin is a highly effective inhibitor of the *in vitro* translation of mRNA. The effect occurs using translation systems prepared from either rabbit or guinea pig reticulocyte lysates. Furthermore, this effect occurs at pharmacologically relevant concentrations, suggesting a possible role of this inhibition in cisplatin action or cytotoxicity. Animals undergoing treatment with therapeutic doses of cisplatin have been found to have tissue concentrations of the drug as high as 230 μM (13, 14). Cisplatin concentrations of 33 μM have been reported in tissue from human patients undergoing treatment with this anticancer agent (15). Inhibition of peptide synthesis *in vitro* is found at concentrations as low as 3 μM with an IC_{50} of 39 μM. Thus, concentrations of cisplatin found to inhibit protein synthesis *in vitro* are on the same order as tissue concentrations found during its therapeutic administration. Also, the highest amount of cisplatin in a cell is found in the cytosol associated with the microsomal fraction (13), which is consistent with cisplatin having an effect on protein synthesis.

Cisplatin inhibition of peptide synthesis can be accounted for by an interaction with mRNA. This hypothesis is based on the following indirect evidence. First, in messenger-dependent assays in which cisplatin is incubated separately with either the mRNA or the lysate, the drug has comparatively little effect on the lysates. Further, in experiments antagonizing the cisplatin effect with dithiobiuret, reversal of the cisplatin effect is observed only when dithiobiuret and cisplatin are combined before the drug is exposed to mRNA. In cases in which dithiobiuret is added after template and cisplatin have been combined, inhibition is not diminished. The possibility of cisplatin binding to mRNA is not surprising as it has previously been shown to bind to nucleic acids (2–5).

This effect of cisplatin cannot be explained by digestion of the message by either cisplatin itself or by some contaminant of the drug solution. Inhibition of *in vitro* translation occurs in the presence of RNasin, a specific inhibitor of ribonucleases. Furthermore, agarose gel electrophoretic analysis of mRNA preincubated with cisplatin shows no degradation or other alteration in the migration of the message at concentrations that result in essentially complete inhibition of peptide synthesis. Reversal of the inhibition by dithiobiuret is evidence that the action of cisplatin is not due to a contaminant in the preparation. Dithiobiuret is shown to react with cisplatin. If a contaminant is present, it would still be added to the mRNA in the translation assay and unless it too is inactivated by dithiobiuret it should inhibit peptide synthesis. This does not occur.

Qualitative differences occur in the peptide products whose translation is directed by cisplatin-treated mRNA. SDS-polyacrylamide gels of products from inhibited *in vitro* translation assays show much greater inhibition of peptides of higher molecular weight than of the synthesis of the smaller products. This same result was observed with both guinea pig and rabbit endogenous mRNAs and viral (BMV) mRNAs exposed to cisplatin. The electrophoretic pattern shown in the fluorograph does not have any novel bands or excessive smearing. This absence of such partial products may suggest that cisplatin acts to inhibit the initiation of translation. Alternatively, partial or incomplete peptides formed may be proteolytically digested in the lysate and so not visible in the SDS-polyacrylamide gels. It has been previously suggested that incomplete peptide products are readily hydrolyzed (16). It is possible that cisplatin may inhibit elongation by interfering with the movement of the ribosomal complex on the mRNA, either by being directly bound or by prevention of secondary structure melting in front of the translocating ribosome.

Inhibition of the formation of specific large peptides may explain the variability in reports on the relationship between cisplatin and protein synthesis *in vivo*. Some investigators found that cisplatin measurably decreased peptide synthesis (8) whereas others did not (6, 7). Cisplatin may inhibit the synthesis of certain large peptides even though overall inhibition of translation may be undetectable. This selective inhibition may lead to toxicity in the cell. We are currently considering several possible mechanisms for this effect. It has been reported that formation of specific intrastrand cisplatin cross-links occur at several sites in DNA, including an adenine that is 5′ to a guanine when separated by a third base (2). If such specific binding occurs to mRNA, then the initiation of peptide synthesis could be blocked by a cisplatin linkage within the translation start codon, 5′-AUG. The greater inhibition of large peptides, however, is not explained by such a mechanism.

A role for mRNA secondary structure in the control of translation initiation has been proposed (17). This was shown by a decrease in translation of an mRNA that contains a mutation that increases the stability of a base-paired hairpin structure of known sequence that includes regions critical for the initiation of translation. A cisplatin cross-link in this region of a mRNA might also interfere with initiation. If the larger

peptides are synthesized from larger mRNA templates and these form a greater number of base-paired hairpin loops, then the greater mRNA size may allow for more cisplatin cross-linking and account for the greater inhibition of large peptides.

Cisplatin cytotoxicity has been shown to be modulated by the protein synthesis inhibitor sparsomycin (6). Sparsomycin inhibits peptidyl transferase associated with the large ribosomal subunit. Sparsomycin (10 μg/ml) potentiates cisplatin cytotoxicity and cisplatin potentiates inhibition of protein synthesis by sparsomycin. This interaction between sparsomycin and cisplatin could occur because the drugs have two different mechanisms of protein synthesis inhibition. Furthermore, low concentrations of sparsomycin (1 μg/ml) afforded significant resistance to cisplatin cytotoxicity. Cells respond to an inhibition of protein synthesis by agents like sparsomycin by temporarily enhancing the biosynthesis of rRNA. If this occurs in the presence of low concentrations of sparsomycin, then protection from cisplatin cytotoxicity may be due to increased binding of cisplatin to the excess RNA being produced. Cisplatin interacting with this excess RNA might reduce its effect on translation.

Acknowledgments

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