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# BIOCHEMICAL STUDIES ON THE POTENTIATION OF ANTITUMOR ACTIVITY OF CISPLATIN BY ADRIAMYCIN

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Summary. The RNA synthesis in vitro by E. coli RNA polymerase was found to be highly sensitive to cis-diamminedichloroplatinum (cisplatin) inhibition. The degree of inhibition was in proportion to the length of time of template preincubation with cisplatin, suggesting that cisplatin-template binding was involved in the inhibition of RNA polymerase. The effect of adriamycin on this inhibition was studied and it was found that adriamycin significantly enhanced the inhibitory effect of cisplatin and that the total effect was greater than the sum of the effects of each drug used individually. This synergistic effect was not observed when the effect of the combination of adriamycin and cisplatin on in vitro DNA synthesis was studied.

cis-Diamminedichloroplatinum (cisplatin[2]) in combination with adriamycin elicits a marked therapeutic potentiation of patient tumor remission, particularly against ovarian carcinoma. Data obtained by summing the results of 7 clinical studies in six institutions demonstrated that cisplatin, when used as the sole agent, results in approximately a 25% response rate of remission against the late stages of the disease (1) while adriamycin alone yields a remission rate of 38% (2). Bruckner et al. (3) have recently reported an 80% remission rate with the cisplatin plus adriamycin combination against ovarian carcinoma patients. The therapeutic results achieved with cisplatin plus adriamycin are greater than the sum of the results expected if each drug were additive, i.e. 63%. Hence, this combination elicits a synergistic effect in its therapeutic efficacy against ovarian carcinoma. Using this same drug combination a similar therapeutic

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[2]The abbreviations used are: cisplatin, cis-diamminedichloroplatinum; MOPS, 3-(N-morpholino)propane sulfonic acid.

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synergism has been observed in vavious animal tumor systems (4). In vitro studies of L1210 cell growth have also shown that the combination of cisplatin and adriamycin results in a potentiation of inhibition. (Harder et al., unpublished data).

The biochemical mechanisms of the cytotoxic activities of cisplatin and adriamycin have been attributed to their abilities to bind to cellular DNA and consequently inhibit nucleic acid synthesis (5-9). However,the inhibition of RNA synthesis in vitro by cisplatin, has not previously been reported. Using the d(A-T)-directed reaction as a model system (10), we describe in this report the possible roles of these agents on RNA synthesis in relation to the observed clinical synergism.

## MATERIALS AND METHODS

Materials: Cisplatin was provided by the Drug Research and Development Branch, National Cancer Institute, Bethesda, MD. Adriamycin (doxorubicin hydrochloride) was manufactured by Farmitalia SPA, Italy, and supplied by Adria Laboratories, Wilmington, DE, or it was obtained from Aldrich Chemical Co., Milwaukee, WI.

Preparation of template-cisplatin complex: The alternating copolymer, poly d(A-T) d(A-T), was used as the template for the present studies. Because cisplatin reacts slowly with DNAs in vitro, initial studies involved measuring the template activities as a function of template-cisplatin preincubation time for both E. coli DNA polymerase I and RNA polymerase assays. In these studies, poly d(A-T)·d(A-T) was dissolved in 5 mM MOPS buffer, pH 7.2 containing 5 mM NaCl and dialyzed against the same solution. The concentration of the template was adjusted spectrophotometrically using 6600 as the molar extinction coefficient at $\lambda_{max}$ of 262 nM. Cisplatin, dissolved in the same buffer, was added to poly d(A-T)·d(A-T) in a 2 ml plastic serum vial to yield a final concentration of 500 μM and various cisplatin/base nucleotide ratios, ranging from 0.0001 to 0.5. The reactions proceeded at 4°C with aliquots being removed at various time periods for the assay of template activities.

RNA polymerase assay: E. coli DNA-dependent RNA polymerase (fraction IV) was assayed in a 0.1-ml reaction mixture containing 50 mM Tris-HCl (pH 7.9), 4 mM $MgCl_2$, 5 mM dithiothreitol, 0.2 mM each of ATP, GTP, and CTP, 0.04 mM UTP and [$^3$H]UTP (600-700 cpm/pmol), and template DNA as indicated. After incubation for 30 min at 37 °C, the reactions were stopped by adding 0.1 ml of cold 0.1 M sodium pyrophosphate (pH adjusted to 7.0) containing RNA, 2 mg/ml, bovine serum albumin, 2 mg/ml, 5 mM UTP, and 30% trichloroacetic acid, 0.5 ml. Acid-precipitable radioactivity was collected on Whatman GF/C filters and washed more than ten times with 5% TCA. Filters were then dried and counted in a scintillation counter.

DNA polymerase assay: E. coli DNA-dependent DNA polymerase I was assayed in a 0.25-ml reaction mixture containing 0.1 mM each of TTP, dATP, dCTP, dGTP plus [$^3$H]TTP (645 cpm/pmol), 16 mM $MgCl_2$, 40 mM glycine-NaOH buffer, pH 8.0, 1 mM dithiothreitol and template as indicated. The incubation was at 37° C for 30 min. The reaction was then terminated by the addition of 100 μg of bovine serum albumin and 0.5 ml of a cold 30% trichloroacetic acid solution. The acid-precipitable radioactivity was collected, washed and counted as above.

## RESULTS

Effect of cisplatin on template activities. The effects of cisplatin on the ability of E. coli polymerases to replicate and transcribe poly d(A-T) d(A-T) as a function of time of preincubation of the template with the drug are shown in Fig. 1. A comparison of the rate of [$^3$H]UMP incorporation (Fig. 1A) with the rate of [$^3$H]TMP incorporation (Fig. 1B) shows that the [$^3$H]UMP incorporation was more sensitive to inhibition than the [$^3$H] TMP incorporation. As shown in Fig. 1A, cisplatin, after 16 days preincubation, inhibited the template activity of poly d(A-T)· d(A-T) as assayed with E. coli RNA polymerase by 50% at a cisplatin/base nucleotide ratio less than 0.005, which is approximately 3 to 5 fold lower than the corresponding value obtained with cisplatin-treated poly d(A-T)· d(A-T) assayed with E. coli DNA polymerase (Fig. 1B). Based on template length, the binding of one molecule of cisplatin to one molecule of poly d(A-T)· d(A-T) could cause the inhibition of transcription by RNA polymerase.

From these studies it is apparent that inactivation of both DNA and RNA polymerase by cisplatin required a preincubation of the drug with template. They also demonstrate that a given degree of template inactivation requires a different cisplatin/nucleotide base ratio for cisplatin or for the different enzymes.

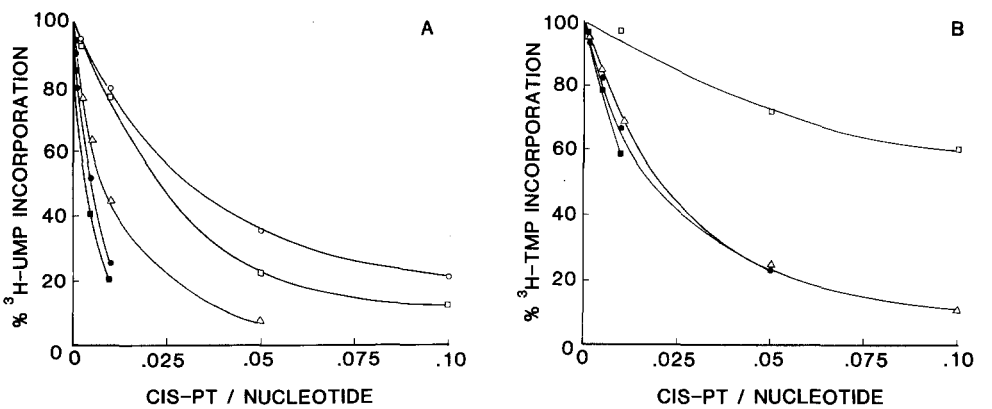


Fig. 1. Template activity of poly d(A-T)·d(A-T) preincubated with cisplatin as assayed by (A) E. coli RNA polymerase or (B) E. coli DNA polymerase I. The assay conditions were as described in Materials and Methods. Preincubation time: ○, 1 day; □, 2 days; △, 7 days; ●, 16 days; ■, 22 days.

TABLE 1

Cisplatin and adriamycin effects on DNA polymerase I activity

| Cisplatin | | Adriamycin | | Cis-Pt + ADM[a] | Potentiation |
|---|---|---|---|---|---|
| Dose (Pt/base) | Inhibition[b] (%) | Dose (μg/ml) | Inhibition[b] (%) | Inhibition (%) | |
| 0.001 | 4 | 4.0 | 23 | 5 | LTA[c] |
| 0.005 | 41 | 4.5 | 17 | 41 | LTA |
| 0.005 | 44 | 4.5 | 28 | 41 | LTA |

[a]Cisplatin plus adriamycin

[b]Each value represents the averagae of results of two to three individual assays.

[c]Less than additive effect

Effects of cisplatin plus adriammycin on template activities. To determine the effect of the combination of cisplatin and adriamycin on template activities, each drug was employed at a single drug dose that resulted in 2-44% inhibition of template activities. For cisplatin, we used platinated templates which had been preincubated for at least 4 weeks. Adriamycin solution was freshly prepared before added to the reaction mixture. Table 1 displays the results obtained with the cisplatin plus adriamycin combination assayed for [$^3$H]TMP incorporation by E. coli DNA polymerase I. It shows that the effects of cisplatin plus adriamycin on [$^3$H]TMP incorporation were less than the combined effects of each drug if used individually. This result was obtained over a large range approximately 4 to 54% inhibition by each drug alone (data not shown). Table 2 shows the results of cisplatin plus adriamycin on [$^3$H]UMP incorporation by E. coli RNA polymerase. In contrast to the effects on in vitro DNA synthesis, this combination, when assayed at the similar range of inhibition by each single drug alone, produced synergistic poteintiaiton against in vitro RNA synthesis.

## DISCUSSION

The cytotoxic effect of platinum compoounds was first demonstrated in the growth of E. coli (11). The antitumor activity of cisplatin was subsequently

TABLE 2

Cisplatin and adriamycin effects on RNA polymerase activity

| Cisplatin | | Adriamycin | | Cis-Pt + ADM[a] | Potentiation |
|---|---|---|---|---|---|
| Dose (Pt/base) | Inhibition[b] (%) | Dose (μg/ml) | Inhibition[b] (%) | Inhibition[b] (%) | |
| 0.00025 | 2 | 3.0 | 18 | 40 | S[c] |
| 0.00025 | 13 | 2.0 | 17 | 42 | S |
| 0.0005 | 17 | 3.0 | 17 | 42 | S |
| 0.0005 | 20 | 4.0 | 17 | 48 | S |
| 0.0005 | 21 | 5.5 | 23 | 53 | S |

[a]Cisplatin plus adriamycin

[b]Each value represents the average of results of two to three individual assays.

[c]Synergistic effect

demonstrated in experimental tumor systems (12). In combination with adriamycin, cisplatin has shown therapeutic potentiation against ovarian carcinoma (3), advanced leukemia and osteogenic sarcoma (4), and L1210 *in vitro* (Harder *et al.*, unpublished data) in comparison with either drug alone. Because both drugs bind to DNA and affect various cellular processes *in vivo* involving DNA as templates, we initiated this investigation by studying the effects of this drug combination on *in vitro* DNA and RNA syntheses.

We have previously shown that adriamycin inhibits RNA syntheses by isolated RNA polymerases (5). The present studies describe a similar inhibition of RNA synthesis by cisplatin. We found that similar to the inhibition by cisplatin of DNA polymerase activity (6), the degree of inhibition of RNA polymerase activity by cisplatin was also in proportion to the length of time of cisplatin-template preincubation, suggesting that drug-template binding was involved in the cisplatin inhibition of RNA polymerase. Furthermore, it is interesting to find that the combination of cisplatin and adriamycin resulted in synergistic (more than additive) effects against *in vitro* RNA, but not DNA, synthesis. Since cancer drugs

that act by different mechanisms may have additive or synergistic therapeutic effects (13), it is attractive to relate the observed synergism to the hypothesis we proposed previously (5) that as far as RNA synthesis inhibitor is concerned, adriamycin needs not function solely as a DNA-binding agent. This hypothesis was further substantiated by our findings (14) showing that non-DNA-binding derivatives of adriamycin inhibit RNA synthesis through drug-RNA polymerase interactions. The fact that without DNA-binding, these derivatives did not affect DNA synthesis (15,16) may account for the observation (Table 1) of the lack of synergism of adriamycin and cisplatin on DNA synthesis. Therefore, synergism may occur as a result of both template and RNA polymerase inactivations.

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