# Some Inhibitory Studies of DNA, RNA and Protein Synthesis in *Escherichia coli* by Platinum Amine Complexes

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## Introduction

Following the earlier observation of Rosenberg *et al.* [1] that *cis*-$Pt(NH_3)_2Cl_2$ (*cis*-PDD) is an effective anti-tumor agent, it was reported that cytological changes were induced in *Escherichia coli (E. coli)* by this drug [2–4]. Inhibition of DNA, RNA and protein synthesis were also reported for a number of platinum containing compounds in both bacteria and eukaryotic cells [5–7].

Explanations of the major changes in anti-tumor activity, with only minor changes in the platinum agent's structure were not easily rationalized [8, 9]. We have investigated the potential inhibitory action on DNA, RNA and protein synthesis in bacterial cell cultures of twelve platinum complexes. From these results, we draw some conclusions concerning the predictability of these new compounds in cancer chemotherapy. Correlations were made between the complexes potency towards the bacteria and their anti-cancer effectiveness.

## Experimental

*E coli* (ATCC 25922) was grown and harvested as previously described [10, 11]. The bacterial cells were suspended in fresh nutrient medium at a density of $3 \times 10^8$ colony-forming units/ml. Bacterial purity and the absence of contamination were established routinely using a grain stain and also by observing colony characteristics on blood agar plates.

Inhibition of bacterial growth was investigated using bacterial suspensions incubated at 37 °C. The

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TABLE I. Complex Abbreviations.

| | |
|---|---|
| 1 | *cis*-dichlorobis(methylamine)platinum(II) |
| 2 | *cis*-dichlorobis(ethylamine)platinum(II) |
| 3 | *cis*-dichlorobis(n-propylamine)platinum(II) |
| 4 | *cis*-dichlorobis(n-butylamine)platinum(II) |
| 5 | *cis*-dichlorobis(isopropylamine)platinum(II) |
| 6 | *cis*-dichlorobis(isobutylamine)platinum(II) |
| 7 | *cis*-dichlorobis(cyclopropylamine)platinum(II) |
| 8 | *cis*-dichlorobis(cyclopentylamine)platinum(II) |
| 9 | *cis*-dichlorobis(cyclohexylamine)platinum(II) |
| 10 | *cis*-dichloro(1,2-diaminobenzene)platinum(II) |

inhibition was then followed by monitoring the absorbance increase at 450 nm as a function of time.

All platinum containing complexes were synthesized by standard methods [12]. These drugs were freshly dissolved in either dimethylsulfoxide (DMSO) or in distilled, deionized water, and they were added to the bacterial suspensions within two hours of dissolution.

Bacteria were first incubated for one hour at 37 °C in the presence of the platinum complexes; DMSO or water were added to control groups of bacteria. Then, 1–2 μCi of [$^3$H]-thymidine, [$^3$H]-uridine or [$^3$H]-leucine were added to both tests and controls [12–14] and radioisotope incorporation was allowed to proceed for 2 h at 37 °C. Five ml of ice-cold trichloroacetic acid (TCA, 10% w/v) containing $10^{-4}$ *M* non-radioactive thymidine, uridine or leucine was added. Samples were refrigerated for 30 min, and the precipitated material was collected on glass fiber filters. The filters were washed and counted using a Searle Liquid Scintillation counter [11, 13, 14].

## Results and Discussion

Previous work [11] demonstrated increased incorporation of thymidine, uridine and leucine into *E. coli* over a period of 3 h at 37 °C. We have now measured the inhibition of DNA, RNA and protein synthesis by the platinum complexes (see Table I) dissolved in DMSO and these results are tabulated in Table II. The inhibition was measured as a function of platinum complex concentration. At the highest dosage, 250 μ*M*, the complexes inhibited all three synthetic processes very effectively and there were little differences in the comparative potencies. However, as the concentration was lowered to 75 μ*M* or 25 μ*M*, differential inhibitory effects were observed. Compounds 1 through 9 had a proportionately lowered inhibitory potency in all three synthetic

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TABLE II. Percent Inhibition of DNA, RNA and Protein Synthesis of Platinun Containing Compounds in DMSO.

| Concentration ($\mu M$)[a] | *Cis*[b] PDD | *Trans* PDD | Compound (See Table I for code) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| DNA Synthesis | | | | | | | | | | | | |
| 250 | 85 | 68 | 88 | 77 | 79 | 99 | 78 | 89 | 90 | 77 | 80 | 100 |
| 75 | 71 | 58 | 37 | 39 | 30 | 37 | 32 | 33 | 36 | 36 | 24 | 92 |
| 25 | 50 | 40 | 36 | 18 | 20 | 24 | 16 | 19 | 27 | 29 | 19 | 86 |
| RNA Synthesis | | | | | | | | | | | | |
| 250 | 50 | 73 | 89 | 86 | 92 | 97 | 73 | 95 | 88 | 89 | 86 | 100 |
| 75 | 82 | 52 | 37 | 17 | 31 | 60 | 26 | 39 | 62 | 28 | 36 | 88 |
| 25 | 30 | 21 | 21 | 11 | 20 | 29 | 18 | 19 | 20 | 16 | 22 | 65 |
| Protein Synthesis | | | | | | | | | | | | |
| 250 | 63 | 53 | 83 | 82 | 88 | 86 | 76 | 80 | 87 | 86 | 75 | 100 |
| 75 | 61 | 52 | 20 | 13 | 15 | 52 | 12 | 42 | 38 | 33 | 35 | 74 |
| 25 | 21 | 13 | 11 | 00 | 7 | 22 | 7 | 11 | 6 | 10 | 3 | 42 |

[a]Actual concentration in contact with bacteria. [b]Each experiment was performed in triplicate.

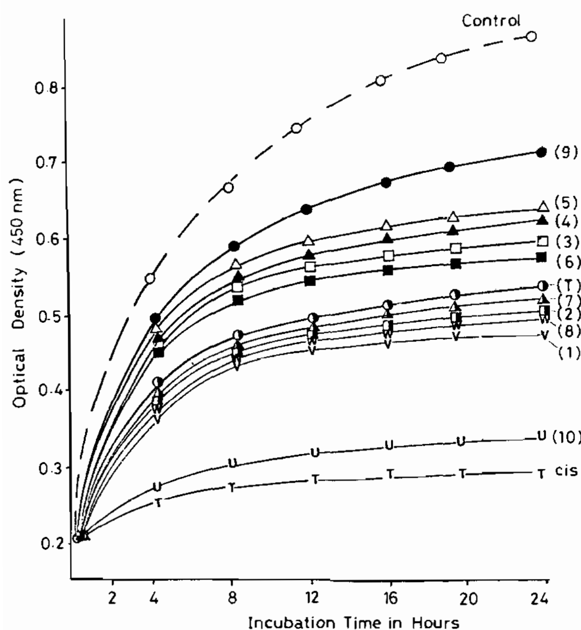


Fig. 1. Growth curves as measured by turbidity of *E. coli* bacteria; grown in nutrient broth. Concentration of platinum complexes dissolved in DMSO is 25 $\mu M$.

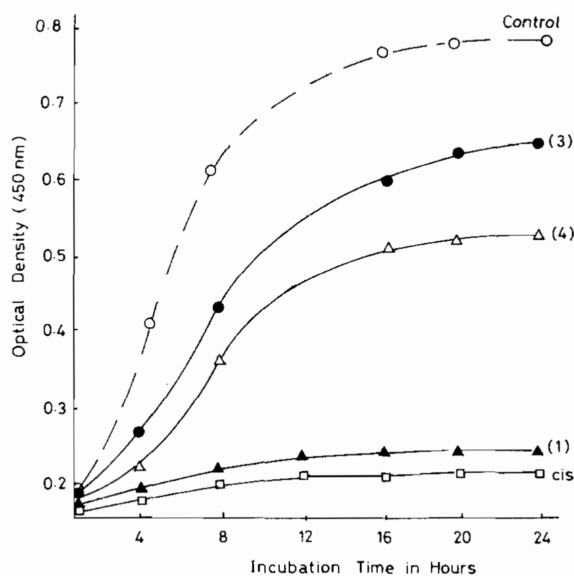


Fig. 2. Growth curves as measured by turbidity of *E. coli* bacteria; grown in nutrient broth. Concentration of platinum complexes dissolved in $H_2O$ is 25 $\mu M$.

systems, while *trans*-PDD, *cis*-PDD and compound 10 showed stronger effects. The potency of these last three complexes was also concentration dependent. It may be noted that complexes 4 and 7 were slightly more effective than the other 7 complexes (not including *trans*-PDD, *cis*-PDD and 10) in inhibiting RNA synthesis suggesting unique interactions during this process.

The time dependence of inhibition by these complexes using DMSO and water as the solvent are illustrated in Figs. 1 and 2, respectively. The choice of complexes for the water study was dictated by their solubilities. There was a rapid cell growth indicated from the increase in turbidity during the first 8 hours. A leveling off took place during the next 8–10 hours after maximum growth was reached.

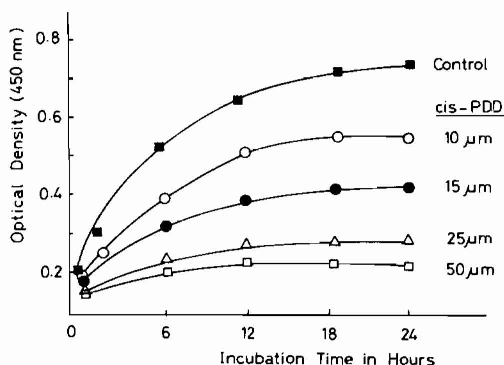


Fig. 3. Inhibition of *E. coli* growth at various concentrations of *cis*-PDD in DMSO.

The inhibitions due to the platinum agents were marked by decreases in this growth. It was observed that the *cis*-PDD and complex 10 had a strong inhibitory effect, several of the other complexes including complex 1 had moderate inhibitory effects in DMSO while *cis*-PDD and complex 10 had a strong effect in water. The concentration dependence of *cis*-PDD on the growth of the *E. coli* in DMSO is recorded in Fig. 3, and it was observed that at 25–50 *μM cis*-PDD, there was a significant decrease in the bacterial growth.

Comparison of bacterial inhibition of synthetic reactions with mammalian anti-tumor activity, expressed as $ID_{90}$ or $LD_{50}$ leads to mixed results. Compounds 4, 5, and 7–9 were found to be potent antitumor agents [15, 16], but yielded weak inhibitions as seen in the present studies. The greatest discrepancy was observed in compound 5 which was a strong inhibitor of mammalian tumor cells. However, compound 10 and *cis*-PDD exerted significant inhibition of bacterial cells as well as the tumor cells. *Cis*-PDD is being marketed as an anti-tumor drug and compound 10 has interesting properties in that it contains a bidentate ligand. Platinum complexes containing these types of ligands merit more comprehensive investigation. The use of DMSO as a solvent for study of the water insoluble complexes also deserves strong consideration since the rates of drug inhibition were similar in both systems.

In conclusion it appears that suppression of bacterial growth is not a reliable predictor of mammalian antitumor effectiveness. Our results also suggest that the antitumor activity of the platinum agents depends upon more subtle factors than just simple inhibition of cellular synthesis of DNA, RNA and protein.

## References

1 B. Rosenberg, L. Van Camp, J. E. Tyosko and V. A. Mansour, *Nature, 222,* 385 (1969).
2 J. A. Howle and G. R. Gale, *J. Bact., 103,* 259 (1970).
3 D. J. Beck and R. R.Brubaker, *J. Bact., 116,* 1247 (1973).
4 B. Rosenberg, E. Renshaw, L. Van Camp, J. Hartwick and J. Drobnik, *J. Bact., 93,* 716 (1967).
5 J. J. Roberts, (Recent Results in Cancer Research Platinum) Coordination Complexes in Cancer Chemotherapy, Springer-Verlag, p. 79 (1974).
6 H. C. Harder, *idem,* 98–112.
7 H. C. Harder and B. Rosenberg, *Int. J. Cancer, 6,* 207 (1970).
8 J. J. Roberts and A. J. Thomson, 'Progress in Nucleic Acid Research and Molecular Biology', Academic Press, New York, Vol. 22, p. 71 (1979).
9 M. J. Cleare, Coordination Chemistry Reviews, Elsevier, Amsterdam (1974): Vol.,12, p. 349.
10 H. H. Kohl, M. E. Freidman, P. Melius, E. C. Mora and C. A. McAuliffe, *Chem. Biol. Interactions, 24,* 209 (1979).
11 H. H. Kohl, S. Haghighi and C. A. McAuliffe, *Chem. Biol. Interactions, 29,* 327 (1980).
12 F. R. Hartley, 'The Chemistry of Palladium and Platinum', John Wiley and Sons, New York, 1973.
13 H. H. Kohl and O. Z. Sellinger, *J. Neurochemistry, 19,* 699 (1972).
14 H. H. Kohl, *Brain Research, 40,* 445 (1972).
15 J. M. Hill, E. Loeb, A. S. Pandue, A. Khan, N. O. Hill, J. J. King and R. W. Hill, Journal of Clinical Hematology and Oncology, Vol. 7, No. 2, Wadley Institutes, Dallas, Texas, 681-699 (1977).
16 Ref. 8. pp. 71–83.