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# BIOCHEMICAL MECHANISMS OF PLATINUM ANTITUMOUR DRUGS

D.C.H. McBrien
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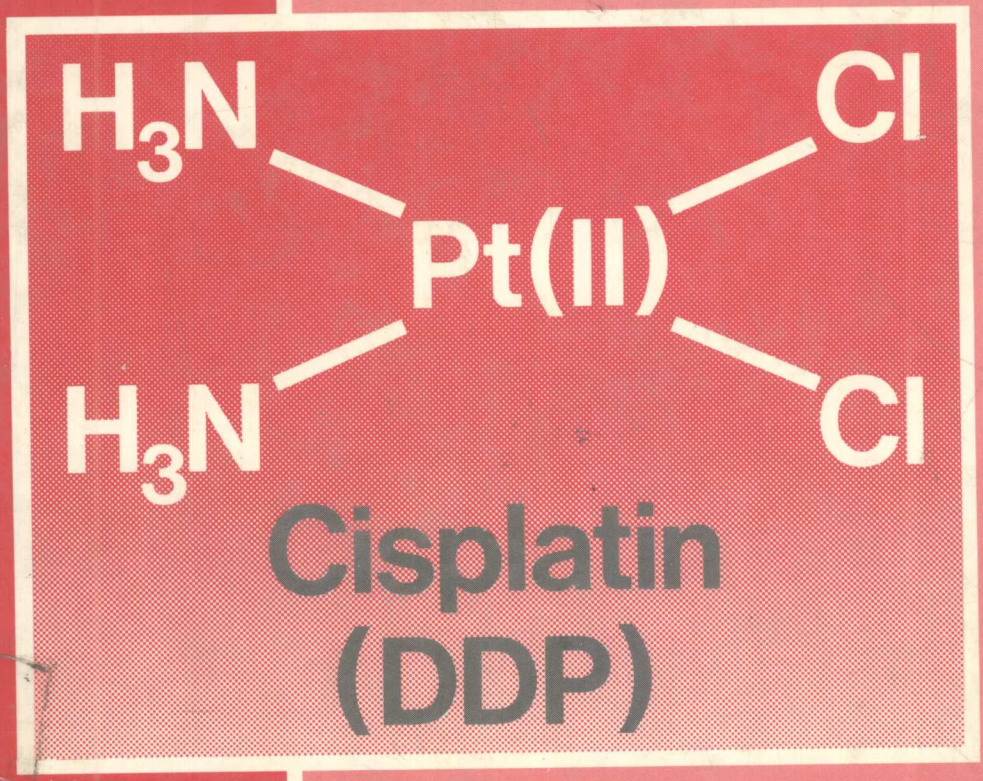


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# BIOCHEMICAL MECHANISMS OF PLATINUM ANTITUMOUR DRUGS

Association for International Cancer Research
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Based on the Proceedings of a Symposium
held at Brunel University, July 3 – 5, 1985

Edited by
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## PREFACE

This book records the proceedings of the fourth symposium on cancer topics sponsored by the Association for International Cancer Research and held at Brunel University on July 3–5, 1985. It is a pleasure, once again, to record our gratitude to our sponsors for their continued support.

The previous volumes in this series were published by Academic Press (London) Ltd., with whom we had a happy working relationship. This volume inaugurates what we hope will be an equally happy and productive relationship with IRL Press, and we are grateful for the enthusiastic help of their staff.

Once again we record our indebtedness to those of our colleagues whose help was essential for the smooth running of the symposium. Principal among these was our fellow member of the Organizing Committee, Dr. K.H.Cheeseman, and we also thank Mrs A.Aboud, Mrs W.Ditschler, Dr. P.Mistry, Dr. S.Flatman, Miss K.Proudfoot, Mrs S.Maddix, Dr. A.Morgan and Mrs J.Delgado.

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## INTRODUCTION

Since cisplatin was licensed for clinical use in 1979 it has become one of the most extensively used of all antitumour drugs. It has had a major success in the control of testicular tumours and is a common component of the drug combinations used in chemotherapy of other tumours (notably those of the ovary). Nevertheless, cisplatin has a number of serious side effects. The dose limiting side effect is nephrotoxicity but some patients find nausea and vomiting so unacceptable that they withdraw voluntarily from potentially lifesaving treatment. In certain new treatment regimens in which the nephrotoxicity is controlled peripheral neuropathy has caused serious and permanent disability in some patients. Cisplatin was the first platinum complex with antitumour properties to be discovered - the story of that discovery by Barnett Rosenberg, a classic case of scientific serendipity, has been told many times and we will not recount it here. It was naturally hoped that a systematic search through platinum complexes would yield other compounds as effective as cisplatin as antitumour agents but with less severe side effects. The search for 'second-generation' platinum antitumour agents was initiated even before the widespread introduction of cisplatin into the clinic. Several of these compounds are, even now, being taken through the final stages of clinical trials - their current status is described in this book by Hilary Calvert.

The search for second-generation platinum analogues, however, was not informed by a detailed understanding of the mechanisms by which cisplatin brought about its various biological effects. Once a few simple rules had been recognised - for example that all antitumour complexes had two relatively labile ligands as leaving groups and these had to have a *cis* configuration - the search had to be conducted on a largely empirical basis. The up and coming second generation compounds are not nephrotoxic but do suffer other dose-limiting side effects and it seems likely that none will succeed in completely displacing cisplatin from the clinic. The introduction of a drug into the clinic before the mechanism of action is understood is a common practice, and, indeed, is perfectly proper. Many millions of people still alive today would by now be dead if they had been deprived of drug treatment whilst mechanistic studies had been performed. However, in the search for more effective analogues of known drugs mechanistic studies come into their own. If we can achieve a fundamental understanding of the biochemical

mechanisms of platinum antitumour drugs it is to be hoped that the chances of developing a new platinum drug with fewer side effects and more desirable properties will be enhanced. It was with this in mind that we conceived the plan for this symposium.

The best-studied aspect of the biochemistry of the platinum drugs is that of their interaction with DNA. Even after nearly two decades of investigation however, it is clear from the discussions and papers in this symposium that there is still some controversy on the nature of the lesions responsible for cytotoxicity and which distinguish the effective antitumour platinum complexes from those complexes which are ineffective. In the first section of this symposium studies on these lesions are described by Neil Johnson and by John Roberts and following that Alan Eastman and Wolter Oosterhuis discuss different aspects of the phenomenon of resistance to platinum drugs exhibited by certain cells *in vivo* and *in vitro*.

It is characteristic of the AICR symposia that time is allowed for extensive discussions. The discussions at this symposium were particularly lively and it is a feature of them that they generated more fundamental questions than answers. Some of these questions are enumerated by the Chairman at the end of the final discussion. One of these concerns whether cisplatin is chemically or metabolically activated in a manner similar to many other toxic agents. Peter Daley-Yates described work with a bearing on this question.

Of the various toxicities of cisplatin none has attracted more attention than the kidney toxicity. In the third section of the symposium Charlotte Jacobs describes work on the secretion of platinum by the kidneys of patients. Rick Borch and David McBrien discuss two methods which have been devised to ameliorate nephrotoxicity and Zahid Siddik compares the pharmacokinetics of the nephrotoxic cisplatin with the non-nephrotoxic second-generation compound carboplatin. Chuck Litterst describes an investigation into the roles of glutathione and metallothionein in determining the renal handling of cisplatin and Bob Safirstein describes the functional changes in the rat kidney induced by cisplatin. In these papers and the associated discussions are a number of unresolved contradictions. To what extent, if at all, is platinum from cisplatin and carboplatin secreted by the kidney and what causes the delay between

exposure to the toxic insult and the onset of pathological symptoms? These questions and others are extensively discussed.

The final section of the symposium concerns itself with analytical techniques of proven and of potential use to workers in this field. Trevor Delves describes ways of overcoming matrix interferences in analysis of platinum by flameless atomic absorption spectrophotometry and introduces a new method of analysis in which inductively coupled plasma emission is used in conjunction with mass spectrometry. Noel McAuliffe describes the preparation of drugs from platinum radioisotopes and Peter Sadler describes the potential of the application of $^{195}$Pt-NMR to the study of platinum drugs and their biological effects. It was our original intention to standardize the abbreviations used for the different platinum analogues. However, it soon became evident that different abbreviations are used by different authors for different purposes (for example for some purposes it is important to stress the *cis* and *trans* in *cis*-DDP and *trans*-DDP, whereas for other authors DDP is a sufficient abbreviation for cisplatin). We therefore abandoned an attempt at standardization and have allowed each author to define his own abbreviations.

**David McBrien**

CONTENTS

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# Biological and biochemical effects of DNA damage caused by platinum compounds

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## INTRODUCTION

DNA is believed to be the cellular target responsible for the antitumour activity of cis-DDP* (Roberts and Thomson, 1979; Roberts and Pera, 1983). However, inactive platinum compounds, such as trans-DDP and [PtCl(dien)]Cl, also enter the tumour cell and bind to the DNA. A number of studies have tried to explain the different biological effects of these platinum compounds by their perturbations of DNA conformation and stability in vitro (for a review see Macquet et al., 1983). The principle effects of these three platinum compounds are summarized in Table 1. The bidentate molecules are easily distinguished from the monodentate compound by the formation of inter- and intrastrand DNA-DNA crosslinks. The most remarkable difference between the two bidentate compounds is that the cis isomer destabilizes DNA while the trans isomer stabilizes the polynucleotide. Although active and inactive compounds have different effects on DNA in vitro, it is difficult to show that these conformational changes are responsible for different perturbations of DNA functions in the cell.

This article will review the alterations of various DNA functions which occur when known amounts of cis-DDP, trans-DDP or [PtCl(dien)]Cl are bound to DNA. In order to compare the

FOOTNOTE

*Abbreviations: DDP, diamminedichloroplatinum(II); $r_b$, molar ratio of bound platinum per nucleotide; en, ethylenediamine; dien, diethylenetriamine.

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Table 1 CHANGES IN THE CONFORMATION AND STABILITY OF DNA AFTER FIXATION OF PT(II)CHLOROAMINES IN VITRO, $r_b$ = 0.01 (AFTER MACQUET ET AL., 1983 AND REFERENCES THEREIN).

| | cis-DDP | trans-DDP | [PtCl(dien)]Cl |
|---|---|---|---|
| DNA chain breaks | 0 | 0 | 0 |
| S1 sensitive single-stranded DNA | 0 | 0 | 0 |
| Interstrand crosslinks (cross-links per Pt) | + (1/30) | + nd | - 0 |
| DNA shortening | + | + | - |
| Exclusion of intercalating agents | + | + | - |
| ΔTm | -2.4 °C | 1.3 °C | 3.3 °C |

effects of different platinum compounds quantitatively, it is necessary to measure their concentrations at the cellular target. Platinum compounds react with most buffers and with nucleophiles in cell culture media and in the cell. The extent of these reactions depends on the platinum compound (Pascoe and Roberts, 1974; Johnson et al., 1980 a,b; Alazard et al., 1982) and platinum binding on DNA is not always a linear function of dose (Alazard et al., 1982) or of time (Johnson et al., 1980 b). As a result there is no simple relation between the concentration of drug which is administered and the

quantity which binds to the DNA. Platinum forms coordinate covalent bonds with the heterocyclic nitrogen atoms of DNA and the platinum-DNA complex can be isolated from treated cells. Platinum fixation on DNA is usually quantified by atomic absorption spectroscopy although it may also be measured using radioisotopic techniques [$^{195m}$Pt] or by means of radioactive ligands such as [$^{14}$C]-en. Immunochemical dosage of platinum-DNA lesions has also been reported (Malfoy et al, 1981: Poirier et al., 1982). DNA binding is usually reported as $r_b$ which is the molar ratio of bound platinum per nucleotide. Alternatively, some authors have considered that the number of platinum atoms per DNA molecule is the biologically relevant quantity (Pascoe and Roberts, 1974).

It is important to keep in mind the different timing of the phenomena which will be examined. Cultured cells or bacteria are generally exposed to drugs for 1-2 h. Although this time represents a fraction of the cell cycle for mammalian cells, bacteria may undergo more than one doubling during treatment. Platinum binding and inhibition of DNA synthesis are usually measured immediately after treatment. Survival is quantified by the formation of colonies 10-20 cell divisions after the measurement of $r_b$ (24 h later for bacteria and 1-2 weeks later for mammalian cells). Mutations are quantified 48 h after treatment of bacteria and 2 weeks after treatment of mammalian cells. The antitumour effect of cisplatin is usually observed more than 1 week after treatment. For example, a drug exhibits an antitumour effect toward L1210 leukemia cells grafted intraperitoneally in mice when the animal survives longer than 11 days. Hence the $r_b$ may not reflect the number of DNA lesions which are present at the time of expression of the biological phenomenon. However, the $r_b$ value immediately after treatment, which corresponds to the maximum density of platinum on the DNA, is probably a good measure of the exposure of the subcellular target to the drug during treatment.

## DNA SYNTHESIS

### In vitro

Experiments which measure DNA synthesis in vitro permit

the manipulation of the DNA template before presenting it to the enzymatic machinery for copying. In the following studies addition of cis-DDP to the enzyme mixture did not significantly inhibit polymerase activity in the experimental conditions used to measure DNA synthesis. Hence the observed inhibition of DNA synthesis can be unambiguously identified with altered template activity due to the presence of DNA lesions.

Giraldi and Taylor (1974) reacted calf thymus DNA with $Pt(en)Cl_2$ and studied its ability to act as a template for DNA sythesis by DNA polymerase from Micrococus lysodeikticus. Thymidine incorporation was decreased by 50% by 3-5 molecules of $Pt(en)Cl_2$ per 100 nucleotides. Harder et al. (1976) studied the template activity of salmon sperm DNA using partially purified DNA polymerase $\alpha$ or $\beta$ from human lymphocytes. Thymidine incorporation was decreased by 50% by cis-DDP at $r_b$=0.01 or 0.015 for polymerase $\alpha$ and $\beta$ respectively. Trans-DDP produced the same inhibition as cis-DDP after fixation of twice as much platinum on the template. These enzymes utilize nicked duplex DNA as a template and primarily fill single-stranded gaps in the double-stranded DNA (Kornberg, 1980). Lesions which occur in the gaps would be expected to inhibit DNA synthesis while platinum which was bound to double stranded DNA would have little effect. Therefore a large number of DNA lesions may be necessary to reduce DNA synthesis quantitatively in these two systems.

Johnson et al. (1978) studied the replication of T7 DNA which had been reacted with cis- or trans-DDP. The template was replicated by a cell-free extract made from phage-infected E.coli. In vitro replication of T7 DNA begins primarily at a single initiation site and the products are biologically active. Any DNA lesion which blocked the movement of the replication fork would reduce DNA synthesis. Hence the sensitivity of the in vitro synthesis from a T7 DNA template is probably a good model for the effect of these drugs on replication in vivo. In vitro replication of T7 DNA was inhibited 63% by cis-DDP at $r_b$ $3 \times 10^{-4}$ which corresponds to 24 platinum atoms per DNA molecule. Trans-DDP was five-fold less inhibitory (Fig. 1).