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Toxicity, Mutagenicity and Drug Resistance in *Escherichia coli* Treated with Platinum Antitumor Compounds*

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Abstract

This article reviews studies in *Escherichia coli* of the mechanism of action of platinum antitumor compounds.

Treatment of bacteria by a series of platinum compounds reveals large differences in their toxicity and mutagenicity. The toxicity of platinum compounds appears to be a result of the inhibition of DNA synthesis. Available evidence suggests that the different sensitivity of wild-type bacteria to a series of platinum compounds is not the result of selective binding on DNA or DNA repair. Rather, each compound forms platinum–DNA lesions which block replication in a characteristic manner.

Genetic requirements for mutation induction by platinum compounds in *E. coli* indicate that the SOS response is required for reversion to prototrophy. Mutagenesis and toxicity vary with the genetic background in a similar way for platinum compounds and certain bifunctional alkylating agents.

Bacterial resistance to platinum compounds may arise from several origins. Repair-deficient bacteria are more sensitive to *cis*-diamminedichloroplatinum-(II) (cisplatin) than their wild-type parent. However, additional mechanisms other than DNA repair may also influence the genotoxic effects of cisplatin. We have studied two bacterial strains which show different toxicity and mutagenicity after treatment with this drug.

Introduction

Bacterial studies have played an important role in the discovery of platinum antitumor compounds and in the investigation of their mechanisms of action. Rosenberg reported the antitumor activity of cisplatin (*cis*-diamminedichloroplatinum(II)) [1] after observing filamentation of *Escherichia coli* which had been exposed to neutral platinum chloroamines [2]. Subsequently bacterial studies have contributed to our current understanding of the molecular pharmacology of this drug. For example, the proof that DNA damage is responsible for the pharmacological effect rests in part on the correlation between the antitumor activity of different platinum compounds and their mutagenicity or prophage induction in prokaryotes [3–8]. In addition, studies of mutant bacteria and the use of DNA cloning techniques give the most detailed information currently available on the biochemical pathways which repair DNA damage caused by platinum compounds. Finally, biochemical and genetic experiments may give some insights into the reasons why cells become resistant to cisplatin, an important clinical limitation of this anticancer drug. This article will review some of the bacterial studies using *E. coli* which contribute to our understanding of the mechanism of action of platinum antitumor compounds.

In order to compare the genotoxic effects of DNA damage caused by different platinum compounds, it is important to measure the platinum on the genome after treatment of the bacteria with biologically relevant concentrations of the drugs. DNA binding is identical in repair-proficient or repair-deficient *E. coli* strains [9–12]. However, exposure of bacteria to equal concentrations of various platinum compounds does not always result in an equal number of platinum–DNA lesions. In addition, DNA binding is not necessarily a linear function of treatment dose [10]. Platinum compounds form stable covalent bonds with bacterial DNA *in vivo* and consequently the DNA can be isolated from cells by standard techniques without disrupting the platinum–DNA binding. Platinum can be quantitated using radioisotopes or atomic absorption spectroscopy and the level of binding is usually reported as *rb*, the molar ratio of platinum per nucleotide.

We have compared the genotoxic effects of a series of platinum compounds in *E. coli* at measured levels of DNA binding. Results show significant differences in the toxicity and mutagenicity per DNA lesion (Table I). Altering the geometric isomer from *cis* to

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TABLE I. *rb* Values which Cause 37% Survival of Wild-type *E. coli* AB1157 after 2 h Treatment with Various Platinum Compounds and the Maximum Number of *his* Revertants Observed

Compound[a]	$rb \times 10^4$	Maximum number of *his* revertants $\times 10^8$ (treatment dose μM)	Reference
cis-$[PtCl_2(NH_3)_2]$	2.5	120 (150)	10, 13, 14, 17
trans-$[PtCl_2(NH_3)_2]$	17	10 (80)	10, 13, 14, 17
DEP	1	50 (100)	b
$[Pt(H_2O)_2DACH]^{2+}$	0.5	30 (100)	b
$[Pt(H_2O)_2(CHA)_2]^{2+}$	1.2	0 (80)	b

[a]Abbreviations: DEP (ethylenediamine)dichloroplatinum(II); DACH, 1,2 diaminocyclohexane; CHA, cyclohexylamine. [b]H. Razaka, unpublished results.

TABLE II Concentrations of Platinum Compounds (μM) which Cause 37% Survival of Various Repair-deficient *E. coli* in Complete Media after 2 h Treatment with *cis*- or *trans*-$[PtCl_2(NH_3)_2]$ [10, 17]

Strain	*cis*-$[PtCl_2(NH_3)_2]$	*trans*-$[PtCl_2(NH_3)_2]$
AB1157 (repair proficient)	21.7	73.3
AB1886 (*uvrA6*)	7	32.5
AB2463 (*recA13*)	1.5	16.7
AB2494 (*lexA1*)	6.7	50
AB2480 (*uvrA6 recA13*)	0.8	7.3

trans decreases both effects by an order of magnitude [13, 14]. The monofunctional platinum compound [PtCl(dien)]Cl is not toxic in wild-type bacteria, even though this compound binds to bacterial DNA [10]. The toxicity of a series of *cis*-Pt(II) chloroamines can vary by a factor of 5 (H. Razaka, unpublished). Unlike cisplatin, the bis(cyclohexylamine) derivative is not mutagenic [6] even though both compounds bind to the bacterial genome (Table I). The following sections discuss the biochemical mechanisms which may be responsible for these differences.

Results and Discussion

Toxicity

A number of results indicate that the toxicity of platinum compounds towards *E. coli* is the result of inhibition of DNA synthesis. First, Thd uptake into the acid-insoluble fraction of bacteria treated with cisplatin is inhibited to a greater extent than uptake of Leu or Urd [15, 16], indicating the preferential inhibition of bacterial replication by these drugs Furthermore, mutant bacteria which are deficient in their capacity to repair DNA damage caused by UV light are also more sensitive to *cis*- and *trans*-$[PtCl_2(NH_3)_2]$ than wild-type bacteria [10, 15, 17] (Table II). Finally, a good correlation exists between the loss of viability and the inhibition of DNA synthesis. On one hand, sensitive repair-deficient bacterial strains show less Thd incorporation after exposure to a given concentration of platinum compound than wild-type bacteria [10]. On the other hand, the toxicity of different platinum compounds towards wild-type bacteria follows the capacity of these compounds to inhibit Thd uptake. For example, if Thd uptake is compared at equal levels of DNA binding, $rb = 10^{-4}$, cisplatin inhibits DNA synthesis an order of magnitude more than its *trans* isomer [10] which correlates well with the relative toxicity of the two compounds (Table I). Similarly, these two compounds inhibit the *in vitro* replication of T7 DNA by a crude bacterial extract at *rb* values of 10^{-4} and the *cis* isomer is 5-fold more effective than the *trans* [18]. Hence the greater toxicity of cisplatin may be due to the formation of DNA lesion(s) which block the progression of the bacterial replication fork more efficiently than DNA damage caused by its *trans* isomer. Alternatively, it is possible that the selective repair of certain DNA damage may be responsible for the relative toxicity of the compounds in Table I.

Several pieces of evidence show that platinum–DNA lesions are repaired in bacteria. First, excision repair clearly occurs in bacteria treated with platinum compounds. For example, the loss of platinum from bacterial DNA can be followed during post-treatment incubation in the absence of drugs [19] and bacteria treated with cisplatin or its *trans* isomer undergo repair synthesis [10, 17]. Furthermore, purified uvrABC excinuclease is able to excise DNA lesions caused by cisplatin or its *trans* isomer *in vitro* [20,

21]. Secondly, platinum compounds are known to induce the SOS response in *E. coli*. For example, these drugs cause filamentation [2, 22], the induction of prophage in lysogenic bacteria [23], Weigle reactivation [24], and error-prone repair is believed to be responsible for much of the mutagenicity of these compounds (see below). Finally, post-replication repair [25] and mismatch repair [12] have been observed in bacteria treated with cisplatin, but adaptation repair does not seem to act on platinum–DNA lesions [11].

Nevertheless, selective repair of DNA lesions formed by less toxic compounds does not seem to account for their lower toxicity. Excision repair does not appear to be responsible for the lower toxicity of *trans*-$[PtCl_2(NH_3)_2]$ or [PtCl(dien)]Cl, for example, since much more repair synthesis is observed in bacteria treated with cisplatin [10]. Similarly, bacteria treated with less toxic compounds do not seem to undergo more SOS repair than those treated with cisplatin. For example, Weigle reactivation is induced by cisplatin but not by its *trans* isomer or [PtCl(dien)]Cl [24]. Furthermore, radioimmunological measurement of the recA protein in *E. coli* treated with these compounds reveals that *trans*-$[PtCl_2(NH_3)_2]$ induces 4 times less and [PtCl(dien)]Cl 10 times less recA than cisplatin [26].

To summarize, the greater survival of wild-type bacteria than repair-deficient bacteria after treatment with cisplatin (Table II) is undoubtedly a consequence of removal or inactivation of platinum–DNA damage by DNA repair processes. However, the different toxic effects of a series of platinum compounds in wild-type bacteria (Table I) does not appear to be the result of selective repair of DNA lesions formed by the less toxic compound. Rather, different platinum compounds seem to form DNA lesions with different capacities to block bacterial replication.

Mutagenicity

Cisplatin does not require microsomal activation in the Ames assay [4, 7, 8, 27]. It causes base-pair substitution mutations in *Salmonella typhimurium* [4, 7, 8, 27] and in *E. coli* [3, 11, 28–30]. There is good evidence for substitution hotspots at GAG and GCG sequences on the DNA of *E. coli* which have been exposed to cisplatin [29].

Reversion to prototrophy is entirely blocked in *recA* or *lexA* mutants treated with platinum compounds [19, 28, 31, 32] (Table III), indicating that their mutagenicity in *E. coli* may be the result of the SOS response which is under the control of these genes [33]. A similar requirement for the *recA* gene product was observed for base-pair substitution mutants at known sequences in the *lacI* gene. However, forward mutations at the *lacI* gene are observed in $recA^-$ bacteria [29] and it is possible that selecting mutants by reversion to prototrophy may not measure mutagenicity of these drugs which is independent of the *recA* gene product.

TABLE III. Toxicity, Mutagenicity and Induction of RecA Protein in Various Mutants of *E. coli* AB1157 in Supplemented M63 Media after 2 h Treatment with Cisplatin (Experimental Procedures as in Refs. 10 and 26)

Strain	μM Cisplatin (37% survival)	*his* Revertants $\times 10^8$	RecA Induction
Wild-type	52	150	12.4
umuC	21	1	12.6
uvrA	5	2.3	8
recA	1.5	0	1

umuC and *umuD* are two genes which are induced during the SOS response [33] and may play a role in the mutagenic bypass of DNA lesions [34]. Cisplatin is less mutagenic in *umuC* bacteria even though recA protein is induced. In contrast with Fram and coworkers [30], we found that low mutagenicity in *umuC* mutants was accompanied by enhanced cytotoxicity (Table III).

An enhanced mutagenic effect of cisplatin has been reported for *E. coli* containing the plasmid pKM101 [29, 31, 32] which codes for two gene products analogous to *umuC* and *umuD* [33]. Platinum compounds are not mutagenic in $recA^-$ bacteria containing the pKM101 plasmid. However, mutagenesis has been reported in bacteria containing pKM101 and a defective recA protein which lacks protease activity but is proficient in homologous recombination [32]. The detailed action of the plasmid differs from the *umuDC* gene products [35]. In particular, plasmid-induced mutagenicity has different base-pair specificity and increases the amount of untargeted mutagenesis in bacteria treated with platinum compounds [36].

The mutagenicity of cisplatin is decreased or abolished in *uvr* mutants of *E. coli* [19, 28–31]. Hypermutability produced by the pKM101 plasmid in bacteria treated with platinum compounds also requires the *uvr* gene products [36]. Excision repair may convert certain platinum adducts to premutagenic lesions that require SOS processing to cause mutations. Alternatively, the SOS system may repair lethal lesions thereby permitting the expression of viable mutants. For example, a *uvr*-dependent recombination process is known to repair interstrand crosslinks formed by bifunctional agents [37]. Interstrand crosslinks are also formed by platinum compounds [38–40] and excision of these lesions followed by recombination and/or SOS processing may be responsible for the increased survival and mutation frequency in bacteria treated with cisplatin.

Resistance

The role of DNA repair in the capacity to survive treatment by cisplatin has already been discussed. For example inducible repair processes such as SOS repair might increase the resistance of bacteria to this drug. In addition, we have observed a case of bacterial resistance to cisplatin which does not appear to be the result of DNA repair.

BS21 is a strain of *E. coli* B/r which was isolated by repeated exposure of its wild-type parent, F26, to the alkylating agent MNNG and has been characterized as constitutive for the expression of adaptive repair [41]. BS21 is also more resistant and less mutable by cisplatin treatment than F26. Cisplatin binds to the DNA of BS21 and F26 with the same efficiency, which shows that impaired transport or sequestration of platinum by reaction with intracellular thiols [42] were not responsible for this phenomenon [11]. The increased resistance of BS21 was independent of the genes *polA*, *uvrA* and *recA* (Table IV) and adaptation repair [11]. *polA* and *ada* affect neither the extent nor the difference in the mutagenesis while *uvrA* and *recA* suppress mutagenesis in both strains. Excision of platinum from treated bacteria was followed during post-treatment incubation in drug-free media by measuring platinum on the DNA with atomic absorption spectroscopy. BS21 and F26 show little excision repair during the 6 h following treatment, F26 being the most efficient of the two [19]. Hence, these biochemical and genetic studies reveal no difference in the capacities of these two strains to repair DNA damage caused by cisplatin. In contrast, both strains are transformed to the same extent by pBR322 DNA which had been treated with cisplatin *in vitro* (Table V).

TABLE IV. Comparison of the Toxicity and Mutagenicity of Repair-deficient Mutants of F26 and BS21 after Treatment for 2 h with Cisplatin [19]

Mutation	Cisplatin (μM)	Survival (%)		*his* Revertants x 10^8	
		F26	BS21	F26	BS21
Repair proficient	66	0.04	0.5	500	40
polA	33	0.04	0.6	400	50
uvrA	13	0.07	0.5	70	25
Δ*recA*	2	0.1	0.4	0	0

TABLE V. Transforming Efficiency of pBR322 DNA Treated *In Vitro* with Cisplatin [19]

Cisplatin (μM)	*rb* x 10^4	Percentage transformation compared with untreated DNA	
		F26	BS21
33	2.4	25	18
83	5.2	4	5
167	10.4	1.5	2

Similarly, Roberts and Rawlings have reported sensitive and resistant lines of Walker cell carcinoma which have identical capacities to excise platinum–DNA lesions [43]. As for F26 and BS21, the two mammalian cell lines are transformed to the same extent by plasmid DNA which was platinated *in vitro* (Roberts, personal communication).

Several explanations of this phenomenon can be imagined. First, the DNA adducts which are formed during platination of the plasmid *in vitro* may not correspond to those formed *in vivo*. For example, under the conditions in which the plasmid DNA was treated for the data in Table V, only 2–10 platinum lesions were present per plasmid, and DNA crosslinks, which account for less than 5% of the platinum–DNA lesions [40], may not be present. On the other hand, plasmids were inactivated at *rb* values of the order of 10^{-4}, the same levels of DNA binding which kill bacteria. In addition the transforming ability of platinated pBR322 decreased in *uvrA* bacteria [19] suggesting that damage on the plasmid DNA is repaired by the same biochemical processes as platinum adducts on the bacterial genome.

Secondly, F26 and BS21 strains may differ in their capacity to tolerate platinum–DNA lesions during replication. A similar effect has been observed in mammalian cells where qualitative and quantitative differences occur in DNA synthesis during the S phase following treatment of sensitive and resistant Walker cells [44]. However a mechanism which permits the bypass of a lesion during replication might be expected to increase the mutation frequency. In contrast, cisplatin is less mutagenic in BS21 than F26.

Finally, the formation of different DNA lesions in sensitive and resistant cells may be considered as a potential mechanism. No difference in the repair processes of BS21 and F26 were observed which could explain their different sensitivity and mutability by cisplatin. When identical platinum–DNA lesions were presented to the bacteria via pBR322, they were processed in the same manner by both strains. These results suggest that cisplatin may form qualitatively different DNA adducts in BS21 and F26 which are responsible for the observed differences in their toxicity and mutagenicity.

Conclusions

Platinum compounds produce DNA damage which kills bacteria by blocking replication. DNA lesions formed by less toxic compounds do not seem to be selectively repaired. Rather, different compounds appear to form platinum–DNA adducts with differ-

ent capacities to inhibit DNA synthesis. Mutation induction by platinum compounds varies with the genetic background of bacteria in a way which suggests that interstrand crosslinks may be the lethal and mutagenic lesion. Although the genotoxic effects of platinum compounds may depend on DNA repair processes, we report one example of a bacterial strain which is resistant to the toxic and mutagenic effects of cisplatin in a way which depends neither on the number of platinum–DNA lesions nor their repair.

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