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## Studies on mutagenesis and repair induced by platinum analogs

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### Summary

Mutagenesis and cytotoxicity were studied in *Escherichia coli* by iproplatin and carboplatin, two analogs of cisplatin (CDDP) currently undergoing clinical trial. As with CDDP, mutagenesis by these agents was mediated by the *umuDC* gene product. In contrast to CDDP, however, mismatch repair did not substantially contribute to survival of cells after exposure to these agents since *dam-3 E. coli* were not more sensitive than wild type *E. coli*. *UvrA*<sup>-</sup> *E. coli*, however were more sensitive to these analogs demonstrating that as with CDDP, *uvr* endonuclease-mediated excision contributes to the repair of DNA damage induced by platinum compounds.

CDDP (*cis*-diamminedichloroplatinum(II)) is an effective agent in the treatment of a broad spectrum of neoplasms (Wolpert-DeFilippes, 1979; Einhorn et al., 1977). An attempt to develop less toxic platinum analogs prompted the synthesis of two compounds: iproplatin (*cis*-dichlorotrans-dihydroxo-*cis*-bis-(isopropylamine) platinum(IV) and carboplatin (diammine (1,1-cyclobutanedicarboxylato)platinum(II)) which are now being evaluated in clinical trials (Wooley et al., 1984; Pen-

dyala et al., 1984). The structures of these compounds are shown in Fig. 1.

CDDP belongs to a group of agents which are SOS mutagens. *Uvr* endonuclease-mediated excision repair contributes to the repair of CDDP induced DNA damage since *uvrA*<sup>-</sup> and *uvrB*<sup>-</sup> *E. coli* are more sensitive to CDDP than wild-type cells (Beck and Brubaker, 1973; Brouwer et al., 1981; Konishi et al., 1981). *RecA*<sup>-</sup> cells are also markedly sensitive to CDDP, although the mechanisms underlying the marked sensitivity of those cells are not as clear as for *uvrA*<sup>-</sup> and *uvrB*<sup>-</sup> cells: *recA* may be required for the repair of cross-linked DNA occurring after CDDP exposure by enhancing recombinational events as well as by its role in regulating the SOS response (Beck and Brubaker, 1973; Brouwer et al., 1981; Konishi et al., 1981). Further, *recA*<sup>-</sup> *E. coli* were not mutable by CDDP (Konishi et al., 1981), indicating that error-prone repair is required for mutagenesis.

We have recently observed that *dam-3 E. coli*,

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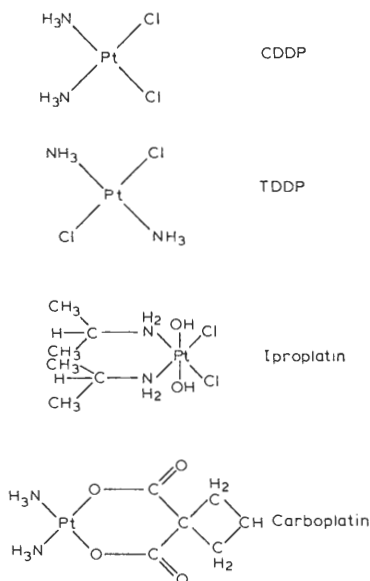


Fig. 1. Structures of CDDP, iproplatin, carboplatin and TDDP.

which engage in aberrant repair of mismatched bases or their equivalent are markedly more sensitive to CDDP than wild-type *E. coli*. Enhanced cytotoxicity of *dam-3 E. coli* was associated with a significant delay in excision of platinum from the DNA of these bacteria (Fram et al., 1985). In addition, we recently clarified the role of the SOS response in CDDP induced mutagenesis by demonstrating that *umuDC* gene product mediates mutagenesis by this agent.

In the present communication, we wished to clarify the role of SOS-dependent mutagenesis by two platinum compounds now undergoing clinical trial as well as by TDDP (*trans*-diamminedichloroplatinum(II)). We also assess the role of mismatch repair and *uvr* endonuclease-mediated excisional repair with respect to cytotoxicity and mutagenesis by these agents.

## Materials and methods

### Bacterial strains

GM 112 (*dam*<sup>+</sup>, wild type *E. coli* K-12) and GM 113 (*dam-3*) are described elsewhere (Bale et al., 1979). GW 2100 (*umuC:Tn5*) was a gift from Dr.

Graham Walker (M.I.T.) AB1157 (*uvrA*<sup>+</sup>, wild-type *E. coli*) and AB2500 (*uvrA*<sup>-</sup>) are described elsewhere (Howard-Flanders et al., 1966). Complete media consisted of Difco brain heart infusion broth and minimal media (Marinus et al., 1983).

### Chemical reagents

CDDP and TDDP were obtained from Sigma Chemical Corp. (St. Louis, MO). CDDP and TDDP were dissolved in dimethylformamide just prior to use. Iproplatin (*cis*-dichloro-*trans*-dihydroxo-*cis*-bis(isopropylamine) platinum(IV) and carboplatin(diammine(1,1-cyclobutanedicarboxylato)platinum(II)) were a gift from Bistol-Myers Company (Syracuse, NY). These reagents were dissolved in minimal salts just prior to use.

### Cytotoxicity and mutagenesis studies

Cells were grown in brain heart media to mid-exponential phase, harvested, resuspended in minimal salts with 1 mM thymine for 60 min at 37°C. Cells were exposed to drug in the dark for 2 h at 37°C, washed twice, and then plated on brain-heart media. Colonies were counted after incubation overnight at 37°C.

Mutation frequency was assessed by resuspending cells at 10<sup>7</sup>/ml in brain-heart media, incubating overnight at 37°C and then plating onto nutrient agar containing rifampicin (100 µg/ml). Total viable bacteria were determined by plating onto nutrient agar plates. Mutation frequency was equal to the number of rifampicin-resistant (*rif*<sup>R</sup>) mutants per 10<sup>8</sup> viable cells.

## Results

The contribution of *umuDC* gene product to mutagenesis by iproplatin, carboplatin, and TDDP is assessed in Table 1. As with CDDP, a marked reduction in mutation frequency by these agents occurs in *umuDC*<sup>-</sup> *E. coli*. Low mutation frequency in *umuDC*<sup>-</sup> *E. coli* was not associated with enhanced cytotoxicity by CDDP or iproplatin in this strain compared to wild-type (Fram et al., 1985). Mutation frequency by CDDP was not significantly higher than with iproplatin (see Table

TABLE 1

MUTATION INDUCTION TO RIFAMPICIN RESISTANCE BY PLATINUM ANALOGS IN *umuDC*<sup>-</sup> *E. coli*

## (A) Mutation frequency after exposure to CDDP

$\mu\text{M}$ CDDP	<i>umuDC</i> <sup>+</sup>	<i>umuDC</i> <sup>-</sup>
0	0 $\pm$ 0 (3)	0
10	60 $\pm$ 16.4 (3)	1
20	107 $\pm$ 31.3 (3)	1
40	218 $\pm$ 169 (3)	1 $\pm$ 1.4 (2)
80	243 $\pm$ 165.6 (2)	15 $\pm$ 0.7 (2)

## (B) Mutation frequency after exposure to iproplatin

$\mu\text{M}$ Iproplatin	<i>umuDC</i> <sup>+</sup>	<i>umuDC</i> <sup>-</sup>
0	0 $\pm$ 0 (5)	1.5 $\pm$ 2.1 (2)
10	152 $\pm$ 91.7 (5)	3 $\pm$ 1.4 (2)
20	159 $\pm$ 14 (2)	2
30	338	0
40	172 $\pm$ 86 (2)	2
100	305	1
300	258	1

## (C) Mutation frequency after exposure to carboplatin

$\mu\text{M}$ Carboplatin	<i>umuDC</i> <sup>+</sup>	<i>umuDC</i> <sup>-</sup>
0	0 $\pm$ 0 (2)	6 $\pm$ 8.4 (2)
100	0.5 $\pm$ 0.7 (2)	0
300	28	2
800	13.3 $\pm$ 8.4 (3)	6
1000	32	7
3000	8	0
3200	100.5 $\pm$ 60.1 (2)	7
6400	504	1

## D. Mutation frequency after exposure to TDDP

$\mu\text{M}$ TDDP	<i>umuDC</i> <sup>+</sup>	<i>umuDC</i> <sup>-</sup>
0	2	1
10	20	-
20	2.5 $\pm$ 2.1 (2)	1
40	7 $\pm$ 8.4 (2)	3
100	7.5 $\pm$ 7.7 (2)	7

Results represent rifampicin-resistant mutants/ $10^8$  viable cells. Each determination represents the mean of duplicates except where the mean  $\pm$  standard deviation and number of independent observations (indicated in parentheses) are shown. The standard deviation of duplicate values is less than 10% of the mean.

1), although both compounds are clearly more mutagenic at a given molar concentration than carboplatin or TDDP. Carboplatin, however, at higher concentrations that are cytotoxic is as

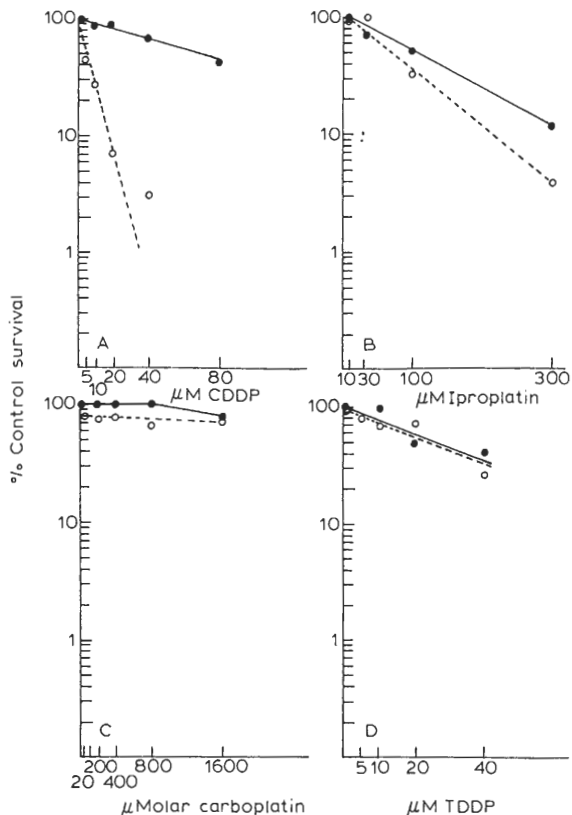


Fig. 2. Cytotoxicity by CDDP and platinum analogs in *dam-3* and wild-type *E. coli*. Cells were exposed to CDDP (A), iproplatin (B), and carboplatin (C), and TDDP (D) for 2 h at 37°C, washed twice, and plated on nutrient agar. Closed circles represent wild-type *E. coli*, while *dam-3 E. coli* are symbolized by open circles. A and D are from the data of Fram et al. (1985).

mutagenic as CDDP and iproplatin (see Table 1 and Fig. 3B). TDDP was minimally mutagenic in both *umuDC*<sup>+</sup> and *umuDC*<sup>-</sup> cells.

Because mismatch repair contributes to the repair of CDDP induced DNA damage in *E. coli*, cytotoxicity by the various platinum analogs was assessed in *dam-3 E. coli*. *Dam-3 E. coli* are not markedly more sensitive than wild-type *E. coli* to iproplatin and both *dam-3 E. coli* and wild-type *E. coli* demonstrated only minimal sensitivity to carboplatin after a 2-h exposure at concentrations up to 1600  $\mu\text{M}$  (Fig. 2).

The cytotoxic and mutagenic effects of the various platinum compounds are shown in Fig. 3.

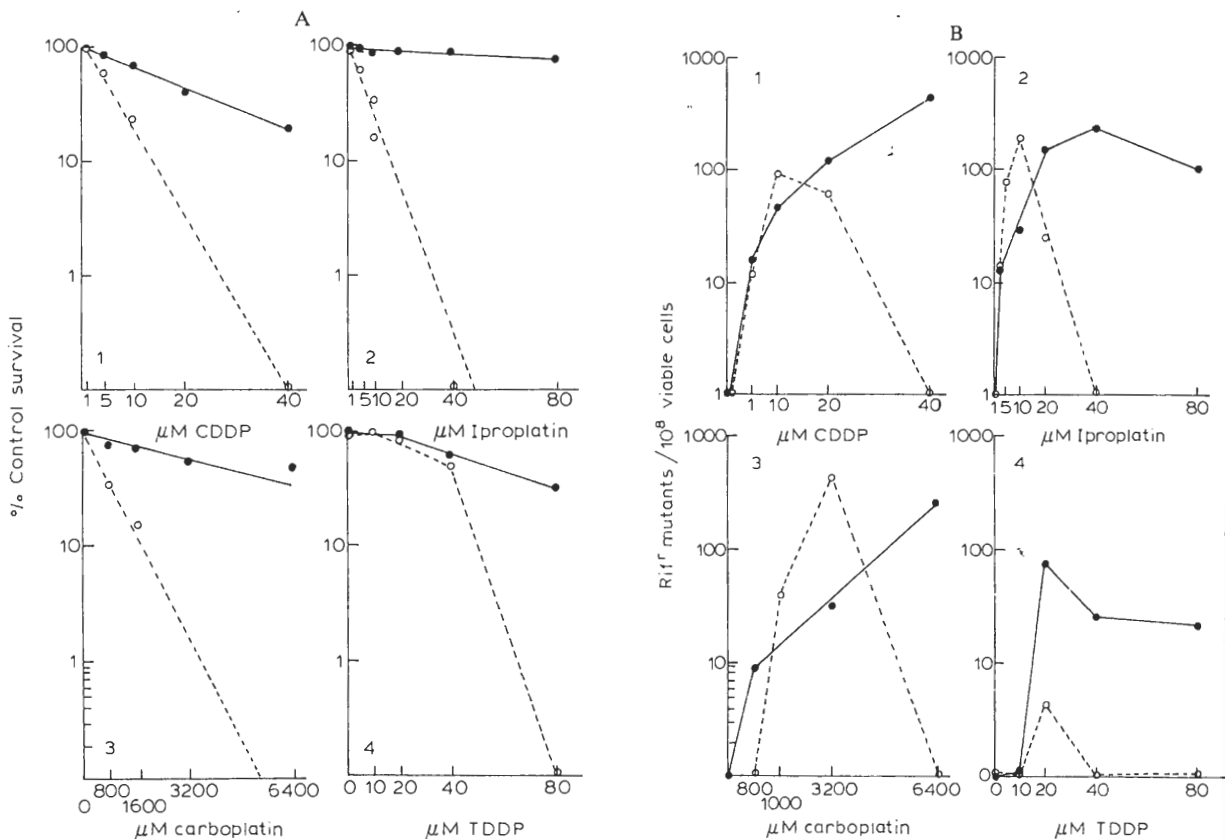


Fig. 3. Cytotoxicity and mutation frequency by CDDP and platinum analogs in *uvrA*<sup>-</sup> and *uvrA*<sup>+</sup>, *E. coli*. Cells were exposed to CDDP (A1, B1), iproplatin (A2, B2), carboplatin (A3, B3) and TDDP (A4, B4) for 2 h at 37°C, washed twice, and either plated on nutrient agar to assess cytotoxicity or grown overnight at 37°C and plated on rifampicin plates to assess mutation frequency. Open circles and dashed lines represent cytotoxicity and mutation frequency in *uvrA*<sup>-</sup> cells while darkened circles and solid lines represent cytotoxicity and mutation frequency in *uvrA*<sup>+</sup> cells.

*UvrA*<sup>-</sup> cells were consistently more sensitive to these agents than *UvrA*<sup>+</sup> *E. coli*. Mutation frequency, on the other hand, was comparable in *uvrA*<sup>-</sup> and wild-type cells at low drug concentrations and reduced at higher drug concentrations in *uvrA*<sup>-</sup> cells.

## Discussion

The involvement of SOS mediated error-prone repair in the induction of mutation by CDDP occurs in both *Salmonella* and *E. coli* (Beck et al., 1973; Brouwer et al., 1981; Konishi et al., 1981). We recently clarified the role of SOS-mediated error-prone repair in CDDP induced mutagenesis by observing a marked reduction in mutation fre-

quency by this agent in *umuDC*<sup>-</sup> *E. coli* (Fram et al., 1985). Iproplatin and carboplatin share with both CDDP and ultraviolet irradiation, a requirement for *umuDC* gene product for mutation induction. Because little is known biochemically with respect to *umuDC* gene product, it is difficult to speculate on the nature of the interaction of this gene product with DNA lesions caused by these agents.

In contrast to CDDP, iproplatin did not markedly enhance cytotoxicity in *dam-3 E. coli*. This finding suggests that aberrant mismatch repair is not induced by this agent to the same extent as with CDDP; an effect likely resulting from structural considerations. The addition of isopropyl groups as well as axial hydroxyl groups

about platinum (see Fig. 1) may sterically alter the binding of this agent to bases in DNA and ultimately influence both the type and quantity of lesions it causes in DNA. For example, octahedral platinum (IV) compounds, such as iproplatin, in contrast to planar platinum (II) compounds, such as CDDP, cause breakage of covalently closed circular PM-2 DNA (Mong et al., 1980). The importance of structural considerations is also shown by the lack of enhanced cytotoxicity in *dam-3 E. coli* after exposure to carboplatin and TDDP (Fram et al., 1985).

CDDP as well as all the platinum analogs tested were more cytotoxic in *uvrA*<sup>-</sup> *E. coli* than wild-type bacteria. This finding demonstrates that *uvr* endonuclease-mediated excision is involved in the repair of DNA damage caused by these agents. Mutation frequency in *uvrA*<sup>-</sup> bacteria was comparable to wild-type bacteria at lower drug concentrations and markedly reduced at higher drug concentrations. This result most likely reflects enhanced cytotoxicity by these agents at higher concentrations in *uvrA*<sup>-</sup> cells and confirms that cells lacking endonuclease are not hypermutable.

The degree of cytotoxicity caused by these agents on a molar basis was: CDDP > iproplatin > TDDP > carboplatin in both *uvrA*<sup>-</sup> and wild-type *E. coli*. This pattern indicates that both the type and configuration of the chloride (or their equivalent) leaving groups are of great significance with respect to cytotoxicity. A prior study has shown that the degree of inhibition of DNA synthesis by CDDP and other platinum analogs was correlated with survival in both wild-type and *uvrA*<sup>-</sup> cells (Alazard et al., 1982). TDDP adducts that are not excised, inhibit DNA synthesis less than CDDP adducts and subsequently *uvrA*<sup>-</sup> cells are less sensitive to TDDP than CDDP. Similar structural considerations may underlie the degree of sensitivity of *uvrA*<sup>-</sup> cells to iproplatin and carboplatin. Thus, wild-type and *uvrA*<sup>-</sup> cells are both more sensitive to the former than the latter agent.

When assessed on a molar basis, CDDP and iproplatin are more mutagenic than carboplatin and TDDP. However, when mutation frequency is analyzed at concentrations of drug that cause com-

parable cytotoxicity, the mutation frequency of the 3 compounds is not significantly different except when compared to TDDP which is minimally mutagenic at all concentrations. These findings suggest that a *cis* configuration is important in mutation induction and are in concert with prior studies (Beck et al., 1973; Mattern et al., 1982).

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