Eur J Cancer Clin Oncol, Vol. 25, No. 1, pp. 27–33, 1989.
Printed in Great Britain

0277-5379/89$3.00 + 0.00

The Mutagenic and Carcinogenic Properties of Three Second Generation Antitumour Platinum Compounds: a Comparison with Cisplatin

RAKESH CHIBBER* and MURIEL J. ORD*

Department of Biology, Medical and Biological Sciences Bldg, Southampton University, Southampton, Hants. SO9 3TU, U.K.

Abstract—*The cytotoxicity, mutagenicity and transforming potentials of three second generation platinum compounds have been investigated in mammalian cells. All the compounds showed positive response in two assay systems in Chinese hamster V 79 cells, i.e. measurement of mutation induction at the HGPRT locus and of DNA damage as indicated by sister chromatid exchange frequencies. At equitoxic doses, the compounds in order of decreasing mutagenicities were cisplatin, spiroplatin, carboplatin and iproplatin. The BHK transformation assay reflected a similar order in the potential carcinogenicity of the drugs. Cisplatin was highly carcinogenic, followed by spiroplatin. In comparison, carboplatin and iproplatin were potentially weak carcinogens.*

INTRODUCTION

THE THERAPEUTIC POTENTIAL of cisplatin is currently well established and has led to its incorporation into first-line chemotherapy regimes for an increasing number of tumour types [1]. Its extensive use has been limited, however, by its toxic side-effects in patients [2]. *In vitro* it has proved highly mutagenic [3–6], and is potentially carcinogenic [6]. The toxic problems encountered with cisplatin have led to the development of a number of second generation platinum compounds [7, 8]. Three, carboplatin [*cis*-diammine-1,1-cyclobutane dicarboxylate platinum (II)], iproplatin [*cis*-dichloro bis(isopropylamine) *trans*-dihydroxy platinum (IV)] and spiroplatin [sulphato-1,2-diaminocyclo hexane platinum (II)], have entered clinical trials. The current clinical status of the new platinum drugs recently reviewed by Barnard *et al.* [9], shows that they have comparable activities to cisplatin but are less toxic. However, there is no information as yet on their mutagenic and carcinogenic potential. In view of their approaching clinical use it has become important, therefore, to evaluate the relative mutagenicity and carcinogenicity of these compounds, since they could prove to have highly undesirable side-effects. In the present work we have used cultured mammalian cells to compare the ability of the four compounds to induce mutations, to damage DNA and to transform cells *in vitro*.

Accepted 3 August 1988.
Correspondence and reprint requests to R. Chibber.
*Members of the MRC Toxicology Research Unit, Carshalton, Surrey SM5 4EF, U.K.

MATERIALS AND METHODS

Cell lines

The work described in this paper was carried out with Chinese hamster cells, V79 lung fibroblasts and baby hamster kidney cells BHK21/C13 (Flow Laboratories). V79 cells were routinely grown in monolayer culture at 37°C in Eagle's Minimum Essential medium supplemented with 10% foetal calf serum (FCS), penicillin (100 IU/ml) and streptomycin (100 μg/ml). The BHK cells were cultured in monolayer from frozen stock, in medium supplemented with 20% FCS and 10% tryptose phosphate broth. The BHK cells were used for transformation when approx. 70% confluent. Both cell lines had approximately equal doubling times of 12 h at 37°C.

Cytotoxicity assay

The survival of cells after exposure to various concentrations of the platinum compounds was determined as follows. Exponentially growing cells were harvested by trypsinization and diluted to give 1×10^2 to 1×10^4 V79 cells or 2×10^2 to 2×10^4 BHK cells per 6 cm dish. Twenty-four hours after seeding, when cells were well attached, drug was added to the culture medium for 1 h at 37°C; medium and drug were removed and fresh

medium added. Following incubation for 7 days, colonies were stained with methylene blue and counted. The average plating efficiencies (PE) for V79 and BHK cells were 95 and 30% respectively. The ratio of mean colony yields of treated to untreated cells, the surviving fractions, was calculated.

Mutation assay

The induction of 6-thioguanine (TG) resistant V79 colonies was determined as follows. 5×10^5 cells were plated in 10 cm dishes and after 24 h incubation the cells were treated with various doses of the compounds for 1 h. They were then washed with PBS and harvested. Some of the cells were plated at 2×10^2 cells per 6 cm dish to determine the toxicity of the drugs. The remaining cells were plated at 5×10^5 cells per 10 cm dish to allow full expression time. Regular subculturing was carried out every 48 h and on day 6 after treatment the cells were plated at 2×10^2 cells per 6 cm dish to determine PE. The remainder were plated at 2×10^5 cells per 10 cm dish (5 plates/dose) in medium containing 10 μM TG. After 7 days incubation TG-resistant colonies were counted and the mutation frequency calculated.

SCE induction

For SCE analysis, approx. 5×10^5 V79 cells were plated per 10 cm dish, 24 h before a 1 h treatment with various concentrations of the drug. The cells were then exposed to 5-bromo-2-deoxyuridine (10 μg/ml) in medium. The dishes were placed in a light-tight box for 28 h to allow the cells to pass through two cycles of DNA replication. Colchicine (10 μg/ml) was added 2 h before harvesting; chromosome preparations were made and sister chromatids were differentially stained using the method of Alves and Jonassan [10]. The preparations were mounted in permount and the number of SCEs per chromosome were determined on the basis of 25 metaphases for each drug concentration and examined directly under oil immersion (× 100). Chromosomes were photographed at × 50.

Cell transformation

BHK cells at a density of 5×10^5 cells/ml were exposed to the drug for 1 h at 37°C. The cells were then recovered by centrifugation, rinsed with PBS and resuspended in fresh medium. For toxicity assay, an aliquot was diluted and plated at densities ranging from 2×10^2 to 2×10^4 cells per 6 cm dish. The dishes were incubated for 7 days to allow colony formation. For the transformation assay, 2×10^5 cells were plated in 10 cm dishes and cultured for 4 days. The cells were then harvested and resuspended in PBS at 1×10^6 cells/ml by strongly pipetting the suspension several times through a 21 G syringe needle. An aliquot was removed and assayed for plating efficiency by plating 2×10^2 cells per 6 cm dish. For growth in agar, 2×10^5 cells in 2 ml of medium, supplemented with 10% FCS and with 0.35% agar (Bacto-Difco) were gently overlaid on a 4 ml basal layer of 0.6% agar in 6 cm dish. Five replicate dishes were set-up for each drug and incubated for up to 3 weeks. Colonies having a diameter of 0.1 mm or more were counted.

RESULTS

Cytotoxicity of platinum compounds towards V79 and BHK cells

Survival of Chinese hamster V79 and BHK cells as a function of increasing concentrations of the platinum drugs is shown in Fig. 1. For all the

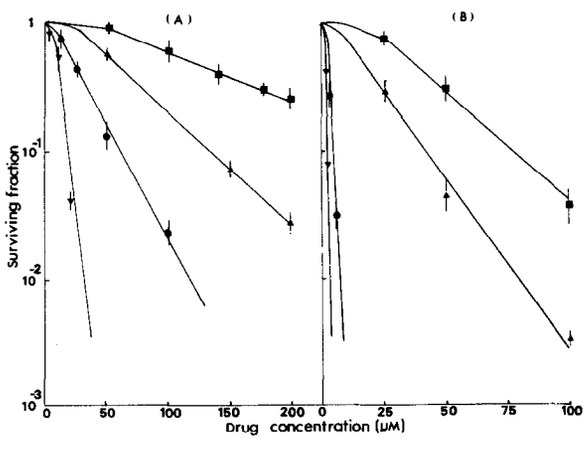

Fig. 1. The effect of various concentrations of platinum compounds on the surviving fractions of (A) V79 cells and (B) BHK cells treated for 1 h at 37°C. (■) Carboplatin, (▲) iproplatin, (●) spiroplatin and (▼) cisplatin. Each point represents the mean of at least three dishes repeated three times; bars = S.E.

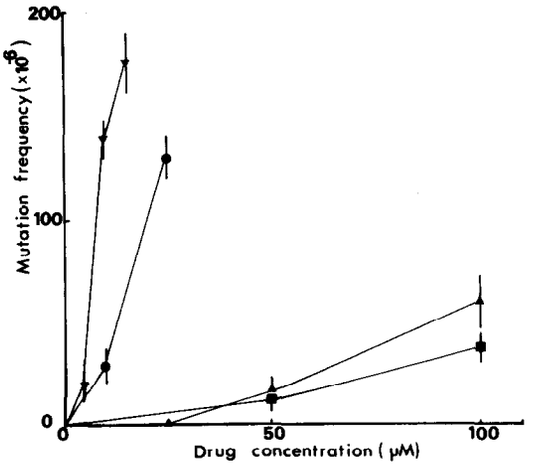

Fig. 2. Induction of 6TG-resistant mutants as function of concentration of platinum compounds. Mutation frequency is expressed as the total number of mutants per 10^6 surviving fraction. (■) Carboplatin, (▲) iproplatin, (●) spiroplatin and (▼) cisplatin. Each point is the mean of five dishes repeated three times; bars = S.E.

Fig. 3. Induction of SCE formation in V 79 cells treated with equitoxic doses (C_0) of platinum compounds. (a) Control, (b) iproplatin, 50 μM (c) carboplatin, 100 μM (d) spiroplatin, 22 μM (e) cisplatin, 7 μM.

compounds the survival curves have a shoulder at low drug concentrations followed by an exponential decrease as a function of increasing concentration in the medium. The shoulder indicates that at lower concentrations the V79 cells can absorb platinum induced damage without expressing lethality. In V79 cells (Fig. 1A) the C_0 values (dose required to reduce survival from a fraction f to $0.37f$ on the exponential part of the survival curve) for cisplatin, spiroplatin, iproplatin and carboplatin were 7, 21, 50 and 100 μM, respectively. BHK cells (Fig. 1B) were approximately four times more sensitive to the toxic effects of platinum compounds than V79 cells. This may reflect differences in drug intake and/or differences in the repair of platinum induced damage in the two cell lines. The different sensitivities cannot be explained by differences in glutathione levels; these were very similar for the two cell lines, i.e. 1.66 nmoles/10^6 cells and 1.92 nmoles/10^6 cells for V79 and BHK cells respectively (Chibber, unpublished data). The C_0 value of cisplatin, spiroplatin, iproplatin and carboplatin in BHK cells were respectively 1, 1.5, 17 and 25 μM, reflecting a similar trend in toxicity of the compounds as in V79 cells.

Mutagenic effects of platinum compounds

The mutagenic activity was measured by the induction of single gene mutation at the HGPRT locus. All the compounds led to an increase in mutation frequency with increasing concentration, with cisplatin the most potent (see Fig. 2). A strong mutagenic response with cisplatin at the HGPRT locus in CHO cells has been reported in earlier studies [4].

Induction of SCE by platinum compounds

DNA damaging effects of the compounds were further investigated using the assay system based on induction of SCEs [11]. Figure 3 shows the induction of SCEs in V79 cells treated for 1 h with equitoxic doses of the platinum compounds. The increase in SCE as a function of molar concentration of each compound is presented in Fig. 4. Background SCE levels in untreated cultures varied from 0.35 ± 0.11 per chromosome. All platinum compounds caused a significant increase. At the maximum concentration tested, cisplatin and spiroplatin caused a 17 and 11 times increase in SCEs over control level, respectively. In comparison, treatment with iproplatin and carboplatin led to only a 4 and 6 times increase in SCEs over control levels respectively. Table 1 compares the SCE and mutagenicity data directly at the equitoxic dose, C_0. At this concentration the compounds clearly differ in their abilities to induce SCEs and mutations at the HGPRT locus.

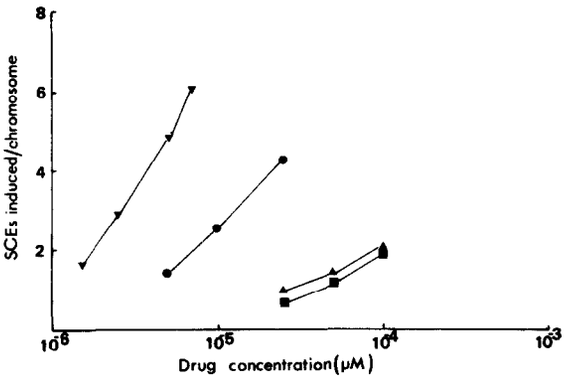

Fig. 4. SCE dose–response curves for the platinum compounds. Cultures were treated for 1 h *with graded concentrations of platinum drugs and the SCE assay was performed.* (■) *Carboplatin,* (▲) *iproplatin,* (●) *spiroplatin and* (▼) *cisplatin. Each represents the mean value of* 25 *metaphase cells from two independent experiments. The standard errors ranged from* 12 *to* 28% *of the mean. Background SCE was subtracted from the mean of treated cells.*

Table 1. *Effects of the platinum compounds in mutagenesis and SCE assays in V* 79 *cells*

Platinum compound	Concentration C_0 (μM)	SCE/chromosome	Mutation frequency per 10^6 cells
Control	0	0.35	2
Cisplatin	7	6.1	140
Spiroplatin	21	4.0	100
Iproplatin	50	1.4	18
Carboplatin	100	2.1	38

Transformation of BHK cells by platinum compounds

Baby hamster kidney cells (BHK) were used as the model system [12] to examine the carcinogenic potentials of the platinum compounds. Table 2 compares the number of transformed colonies found in semi-solid agar following incubation of BHK cells with C_0 doses of each platinum compound. The clone used for these studies had a spontaneous transformation frequency of approx. 50/10^6 cells. Treatment of BHK cells with cisplatin and spiroplatin increased the frequency of induced transformants to 6.2 and 5.2 times the spontaneous transformation frequency. In comparison, carboplatin and iproplatin were less potent and caused a modest 1.7 increase over the spontaneous transformation frequency.

DISCUSSION

Certain drugs used in cancer chemotherapy are known to be mutagenic and carcinogenic in various *in vitro* test systems [13–15]. Some are also known to be carcinogenic in humans as indicated by the development of secondary tumours in patients

Table 2. Comparison of transformation potential of platinum compounds at equitoxic doses (C_0) using a line of BHK cells which had a spontaneous transformation frequency of 50 per 10^6 cells

Platinum compounds	Concentrations C_0 (μM)	Transformation frequency No. of colonies/10^6 cells ± S.E.
Control	0	50 ± 12
Cisplatin	1.0	308 ± 37
Spiroplatin	1.5	258 ± 41
Iproplatin	17.0	81 ± 21
Carboplatin	25.0	86 ± 16

undergoing chemotherapy [16]. There is clearly an important need to investigate the mutagenic and carcinogenic effects of new chemicals being considered for cancer treatment. This report examines the mutagenic and transformation potential of three new platinum compounds undergoing clinical evaluation as anti-tumour agents [9]. The mutagenic potential of cisplatin has been well documented [3–5], but the mutagenicities of the second generation platinum compounds have not been previously reported.

On a molar basis the platinum compounds in the study differ in their toxicity towards V79 and BHK cells, with carboplatin the least cytotoxic in both cell systems. The observed trend in the *in vitro* cytotoxicity of the compounds closely matches their reported toxicity in mice [8].

The mutagenicities of the platinum compounds have been measured by the induction of gene mutation at the V79:HGPRT locus. The induction of SCEs was also evaluated in treated cells. This latter test, which measures sister chromatid exchange frequency, is a reflection of DNA damage and its repair [11]. As such it correlates well with mutagenicity and neoplastic transformation in some cell lines [17, 18]. All the platinum drugs gave a positive response in both assay systems suggesting that they all interact with nuclear DNA. However, the compounds differed in terms of their mutation and SCE induction. When compared at equitoxic doses the compounds in order of decreasing activity are cisplatin, spiroplatin, carboplatin and iproplatin. The 6.2-fold colony increase in the transformation test suggests that cisplatin would be classified as a strong carcinogen (see Styles [12]). Cisplatin is also reported to cause neoplastic transformation of Syrian hamster cells in culture [6] and to induce lung and skin cancers in mice [19]. Spiroplatin also demonstrated carcinogenic activity in the BHK system but carboplatin and iproplatin were relatively weak carcinogens.

In conclusion, the results with the test systems used in this study suggest that the new platinum compounds, carboplatin and iproplatin, which are effective antitumour agents, are less mutagenic and carcinogenic than cisplatin.

Acknowledgements—We wish to thank Johnson Matthey Research Centre for supplying the platinum compounds used in this study.

REFERENCES

1. Prestayko AW, D'Aoust JC, Issell BF, Crooke ST. Cisplatin (*cis*-diamminedichloroplatinum II). *Cancer Treat Rev* 1979, **6**, 17–39.
2. Von Hoff DD, Schilsky R, Reichert CM *et al.* Toxic effects of *cis*-dichlorodiammineplatinum(II) in man. *Cancer Treat Res* 1979, **63**, 1527–1531.
3. Beck DJ, Fisch JE. Mutagenicity of platinum coordination complexes in *Salmonella typhimurium*. *Mutation Res* 1980, **77**, 45–54.
4. Johnson NP, Hoeschele JD, Rahn RO, O'Neill JP, Hsie AW. Mutagenicity, cytotoxicity and DNA binding of platinum(II) chloroamines in Chinese hamster ovary cells. *Cancer Res* 1980, **40**, 1463–1468.
5. Chibber R, Ord MJ. Mutagenic properties of some new anti-cancer platinum complexes. *Human Toxicol* 1985, **4**, 5.
6. Turnbull D, Popescu NC, DiPaolo JA, Myhr BC. *Cis*-platinum diaminedichloride causes mutation, transformation and sister-chromatid exchanges in cultured mammalian cells. *Mutation Res* 1979, **66**, 267–275.
7. Cleare MJ, Hydes PC, Malerbi BW, Watkins DM. Antitumour platinum complexes: relationships between chemical properties and activity. *Biochimie* 1978, **60**, 835–50.
8. Harrap KR, Jones M, Wilkinson CR *et al.* Antitumour, toxic and biochemical properties of cisplatin and eight other platinum complexes. In: Prestayko AW, Crooke ST, Carter SK, eds. *Cisplatin: Current Status and New Developments*. New York, Academic Press, 1980, 193–210.
9. Barnard CFJ, Cleare MJ, Hydes PC. Second generation anticancer platinum compounds. *Chem. Britain* 1986, **22**, 1001–1004.
10. Alves P, Jonasson J. A new staining method for the detection of SCEs in BudR labelled chromosomes. *J Cell Sci* 1978, **32**, 185–195.
11. Wolff S. Measurement of sister chromatid exchanges in mammalian cells. In: Friedberg EC, Hanawalt PC, eds. *DNA Repair: A Laboratory Manual of Research Procedures*. New York, Marcel Dekker, 1981, Vol. 1, 577–585.
12. Styles JA. A method for detecting carcinogenic organic chemicals using mammalian cells in culture. *Br J Cancer* 1977, **36**, 558–563.

13. Benedict WF, Baker MS, Haroun L, Choi E, Ames BN. Mutagenicity of cancer chemotherapeutic agents in the *Salmonella*/microsome test. *Cancer Res* 1977, **37**, 2209–2213.
14. Weisburger H, Griswold DP, Prejean JD, Casey AE, Wood HB, Weisburger EK. The carcinogenic properties of some of the principal drugs used in cancer chemotherapy. *Rec Res Cancer Res* 1975, **52**, 1–17.
15. Singh B, Gupta RS. Mutagenic responses of thirteen anti-cancer drugs on mutation induction at multiple genetic loci and on sister chromatid exchanges in Chinese hamster ovary cells. *Cancer Res* 1983, **43**, 577–584.
16. Arseneau J, Canellas GP, Johnson R, DeVita VT. Risk of new cancer in patients with Hodgkin's disease. *Cancer* 1977, **40**, 1912–1916.
17. Carrano AW, Thompson LH, Lindl PA, Winkler JL. Sister chromatid exchange as an indicator of mutagenesis. *Nature* 1978, **271**, 551–553.
18. Popescu NC, Amsbaugh SC, DiPaolo JA. Relationship of carcinogen-induced sister chromatid exchange and neoplastic transformation. *Int J Cancer* 1981, **28**, 71–77.
19. Leopald WR, Batzinger RP, Miller EC, Miller JA, Earhart RH. Mutagenicity, tumourgenicity and electrophilic reactivity of the stereoisomeric platinum (II) complexes of 1,2-diaminocyclohexane. *Cancer Res* 1981, **41**, 4368–4377.