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Cytogenetic effect of carboplatin on human lymphocytes

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Summary. Carboplatin, a second generation cisplatin analogue, was tested for induction of sister chromatid exchange (SCE) as well as chromosomal aberrations in human lymphocytes in vitro and in vivo. A dose-dependent effect was observed for increased frequency of metaphases with SCE ($r = 0.984$, $P < 0.001$) as well as chromosomal aberrations ($r = 0.994$, $P < 0.001$), primarily chromatid gap or break, in vitro. SCE induction by carboplatin was less than that by cisplatin at the same concentration. When patients were treated with a single dose of carboplatin at a dose of 450 mg/m^2, the frequency of SCE and chromatid type aberrations increased significantly. However, even when considering dose and peak plasma concentration in patients receiving carboplatin, it appears that the ability of carboplatin to induce SCE and chromosomal aberrations is weaker than that of cisplatin. SCE frequencies induced by carboplatin decreased with time going by, and in one patient who was tested 5 weeks after treatment, SCE frequency showed a decrease to the pretreatment level. It thus appears that carboplatin has an improved therapeutic index over the parent compound, cisplatin, because of its less mutagenic or carcinogenic hazard, in addition to the largely reduced incidence of untoward effects.

Introduction

Recently, an increasing number of publications have reported the occurrence of second tumors after successful treatment with chemotherapy and/or radiotherapy for various diseases [8]. During the last decade, the prognosis of patients with small-cell lung cancer has improved with advances in chemotherapy, with 5%–10% of patients currently having a chance of long-term, disease-free survival [5]. These patients are thus considered to have a risk for second tumors, such as leukemia, that may be related to the treatment for cancer. In the recent studies, the cumulative risk of secondary acute nonlymphocytic leukemia and preleukemia reached 14%–25%, especially when alkylating agents were used, 3 or 4 years after the start of chemotherapy for small-cell lung cancer [2, 14]. The combination *cis*-diamminedichloroplatinum (II) (cisplatin) and etoposide has been shown to be effective treatment even in patients failing induction chemotherapy, such as the combination of cyclophosphamide, adriamycin and vincristine [12]. However, cisplatin has severe side effects, such as emesis, renal impairment, and hearing loss. In addition, cisplatin has been shown to induce numerical and structural changes in chromosome, as well as an elevation of sister chromatid exchanges (SCE) [11, 20]. To improve the therapeutic effect and decrease side effects, an intense effort has been made to develop new platinum analogues.

Carboplatin, *cis*-diammine-1,1-cyclobutane decarboxylate platinum (II), is a cisplatin analogue selected on the basis of similar or increased therapeutic effects, compared to the parent compound, cisplatin, and reduced nephrotoxicity in experimental systems [7]. Phase II study of carboplatin has currently been undergone in a variety of cancers, demonstrating that carboplatin is very active in the treatment of small-cell lung cancer, head and neck cancer, and ovarian cancer, with little or no nephrotoxicity and less emesis than cisplatin, although thrombocytopenia was observed but proved reversible in most patients [3, 17].

In the present study, we examined the cytogenetic effects of carboplatin on chromosome of lymphocytes using SCE and chromosomal aberrations both in vitro and in vivo.

Materials and methods

For the in vitro experiments, heparinized blood samples obtained from four healthy donors were cultured in RPMI-1640 medium supplemented with 17% fetal calf serum, penicillin (100 IU/ml), streptomycin (100 μg/ml), and 0.2 ml phytohemagglutinin M (Difco). After 24-h culture, 10 μ*M* 5-bromodeoxyuridine (BrdU) and carboplatin or cisplatin were added to each culture bottle at a final concentration of 0.025, 0.05, or 0.1 μg/ml in normal saline, respectively. Carboplatin (1 or 5 μg/ml) was also tested in two donors. As a control, one culture with normal saline was used. After additional 48-h incubation in dark, highly humidified 5% CO_2 incubator at 37° C, colcemid (0.1 μg/ml) was added to the culture 4 h prior to harvest. Harvested cells were then treated with a hypotonic solution (0.075 *M* potassium chloride) and fixed with methanol/acetic acid (3:1). Slides were prepared by forceful blowing of cell suspensions and were air-dried. The sister chromatids were differentially stained according to the method described by Perry and Wolff [15], with some modification. In each sample, more than 20 metaphases showing differ-

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ences in staining chromatid were photographed and then scored for SCE frequency analysis. The statistical significancy was evaluated using Student's *T*-test and correlation coefficiency.

Parallel cultures were carried out without BrdU for chromosomal aberration analysis. Twenty-four hours after start of the culture, the same concentration of carboplatin or cisplatin used in SCE analysis was added to the culture medium. Cells were harvested after 72-h incubation with 0.1 μg/ml colcemid during the last 4 h of incubation, and were stained using 2% Giemsa solution. Chromosomal aberrations were determined in 100 metaphase cells. Nomenclature for chromosome aberrations was arrived at according to the criteria by the report of the Standing Committee on Human Cytogenetic Nomenclature (1978) [9].

In the in vivo experiments, heparinized blood samples were taken at different intervals, before and after carboplatin administration, from five patients with primary lung cancer who had received no prior chemotherapy and radiotherapy. The dose of carboplatin used in the present study was 450 mg/m^2 i.v. drip infusion (30 min) as a single dose. The blood samples were cultured for 72 h and the rest of the procedure was the same used for the in vitro study.

Results

SCE and chromosomal aberration in healthy donors

The frequencies of SCE in cultured human lymphocytes are shown in Table 1. Both carboplatin and cisplatin caused a significant dose-dependent increase in SCE frequency (r = 0.994 and 0.984, $P<0.001$ for cisplatin and carboplatin, respectively). The increase in SCE frequency after incubation of lymphocytes with cisplatin was greater than that with carboplatin at all concentrations (mean, 2.55-fold). SCE frequency per cell cultured with 0.05 μg/ml cisplatin (28.9 ± 9.2) was similar to that observed in lymphocytes cultured with 1.0 μg/ml carboplatin (27.4 ± 5.5), twenty times the concentration of cisplatin. The degree of chromosomal aberrations increased significantly in lymphocytes treated with more than 1.0 μg/ml carboplatin (Table 2) (Fig. 1). The aberrations observed were mainly

Table 1. Sister chromatid exchange frequencies in cultured human lymphocyte treated with cisplatin or carboplatin in healthy donors

Subject no.	SCE/cell								
	Control	Cisplatin concentration (μg/ml)			Carboplatin concentration (μg/ml)				
		0.025	0.05	0.1	0.025	0.05	0.1	1	5
1.	9.8±2.7	22.1±6.5*	25.9± 7.4*	46.1±13.5*	9.9±3.4	9.9±2.6	13.1±3.4*	ND	ND
2.	8.2±3.0	18.3±5.8*	26.8± 7.4*	40.6± 9.5*	10.0±3.0	11.3±2.7*	11.3±3.1*	ND	ND
3.	9.6±2.3	17.0±4.6*	26.3± 6.5*	43.5±13.4*	11.1±2.7	12.2±4.1**	14.5±2.9*	27.0±5.5*	57.2± 9.5*
4.	7.5±2.5	22.7±5.8*	36.2±10.8*	44.4±10.7*	12.3±4.2*	12.9±2.9*	14.2±3.3*	27.8±5.6*	56.8±15.1*
Mean	8.7±2.8	20.0±6.1*	28.9± 9.2*	43.5±11.7*	10.8±3.6*	11.6±3.4*	13.4±3.4*	27.4±5.5*	57.0±12.4*

Values are mean of more than 20 cells ± SD
Statistical significance by Student's *t*-test compared to the control: **, $P<0.02$; *, $P<0.001$
ND, not done

Table 2. Incidence of chromosomal aberrations induced by carboplatin in cultured human lymphocytes of healthy donors

Subject no.	Carboplatin concentration (μg/ml)	Chromatid			Chromosome			No. of abnormal cells
		Gap	Break	Exchange	Break	Dicentric	Ring	
1.	0	1	0	0	0	1	0	2
	0.05	1	2	0	0	0	0	3
	0.1	2	2	1	0	1	0	4
2.	0	1	0	0	0	0	0	1
	0.025	1	0	0	0	0	0	1
	0.05	1	1	0	1	1	0	2
	0.1	1	1	0	0	1	0	2
3.	0	2	1	0	0	0	0	3
	0.05	2	2	0	0	0	0	4
	0.1	1	4	0	0	1	0	4
	1.0	2	4	0	2	2	0	8
	5.0	14	18	7	3	1	0	26
4.	0	0	0	0	0	0	0	0
	0.05	4	2	0	0	0	0	6
	0.1	1	3	0	0	0	0	4
	1.0	4	4	0	2	2	0	11
	5.0	9	14	8	2	1	1	24

In each culture 100 metaphases were analyzed

Table 3. Sister chromatid exchange frequencies in cultured lymphocytes of patients receiving carboplatin

Patient no.	Age in years	Sex	SCE/cell				
			Days since current therapy	No. of cells examined	Mean ± SD	Range	*P*
1.	64	Male	Before	26	10.8 ± 4.7	3–18	–
			1	51	27.7 ± 5.8	16–44	<0.001
			7	54	17.5 ± 3.8	10–28	<0.001
2.	79	Male	Before	22	6.8 ± 2.5	3–11	–
			1	28	17.3 ± 7.7	6–32	<0.001
			7	27	17.3 ± 3.9	11–25	<0.001
3.	70	Male	Before	26	7.9 ± 2.9	5–14	–
			7	29	12.3 ± 3.8	7–22	<0.001
4.	76	Male	Before	21	6.6 ± 2.3	3–11	–
			1	20	20.5 ± 5.4	11–30	<0.001
			18	21	8.5 ± 3.3	4–15	<0.05
5.	63	Female	Before	23	7.4 ± 1.8	4–10	–
			16	23	12.6 ± 4.3	5–21	<0.001
			35	21	8.9 ± 3.2	3–17	NS

NS, not significant

Table 4. Incidence of chromosomal aberrations in lymphocytes of patients receiving carboplatin

Patient no.	Age in years	Days since current therapy	Chromatid			Chromosome			No. of abnormal cells
			Gap	Break	Exchange	Break	Dicentric	Ring	
1.	64	Before	0	0	0	0	0	0	0
		1	2	2	0	1	1	0	6
		7	1	1	1	0	0	0	3
2.	79	Before	1	1	0	0	0	0	2
		1	2	5	0	1	0	0	7
		7	4	1	1	1	0	0	7
3.	70	Before	2	0	0	0	0	0	2
		7	3	2	0	1	1	1	6
4.	76	Before	1	0	0	0	0	0	1
		1	1	2	0	0	1	0	3
		18	5	2	0	0	0	0	7
5.	63	Before	1	1	0	0	0	0	2
		1	5	6	0	1	0	0	11

In each culture 100 metaphases were analyzed

chromatid-type, such as gaps and breaks, showing dose dependency ($r = 0.994$, $P < 0.001$). A high frequency of chromosomal aberrations was caused by 1 or 5 μg/ml carboplatin, which also significantly increased SCE frequency.

SCE and chromosomal aberrations in patients

SCE frequencies observed in patients receiving carboplatin are shown in Table 3. A significant increase in SCE frequency was observed in all patients after treatment with carboplatin, compared with the pretreatment value. SCE frequency tended to be the highest 24 h after carboplatin administration (Fig. 2), and to decrease to the pretreatment level 5 weeks after carboplatin treatment. Table 4 shows the frequency of chromosomal aberrations in patients treated with carboplatin. An increase in chromosome aberrations was observed in all patients compared with pretreatment frequency, showing mainly increases in chromatid gaps or breaks.

Discussion

Although the mechanism of SCE is not well understood, the induction of SCE is one manifestation of DNA damage, and the ability to induce SCE has been used to identify genotoxic agents that are mutagenically and/or carcinogenically hazardous [10, 19].

In this study, carboplatin showed a significant increase in SCE frequency in human peripheral lymphocyes both in vitro and in vivo. In patients treated with a single dose of carboplatin, there was a reduction in the number of SCE with time, and in one patient, the frequency of SCE returned to the pretreatment level 5 weeks after carboplat-

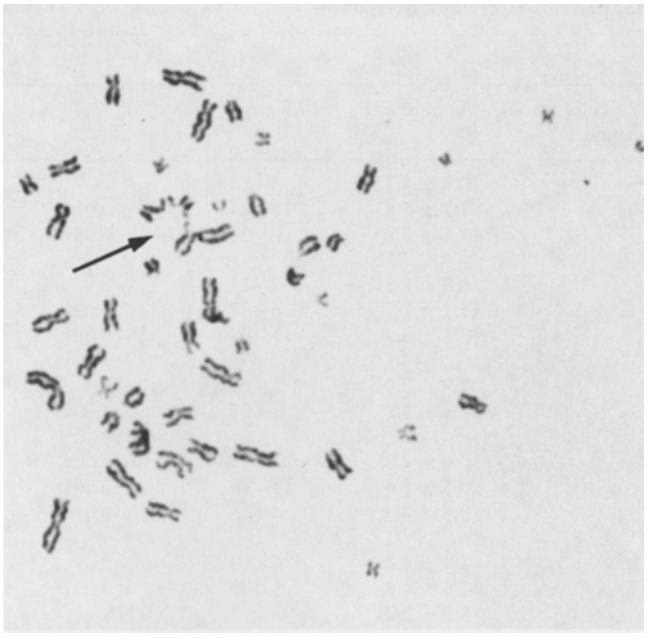

Fig. 1. Metaphase chromosome of lymphocyte treated with 1.0 μg/ml carboplatin. *Arrow* indicates a complex and incomplete chromatid exchange

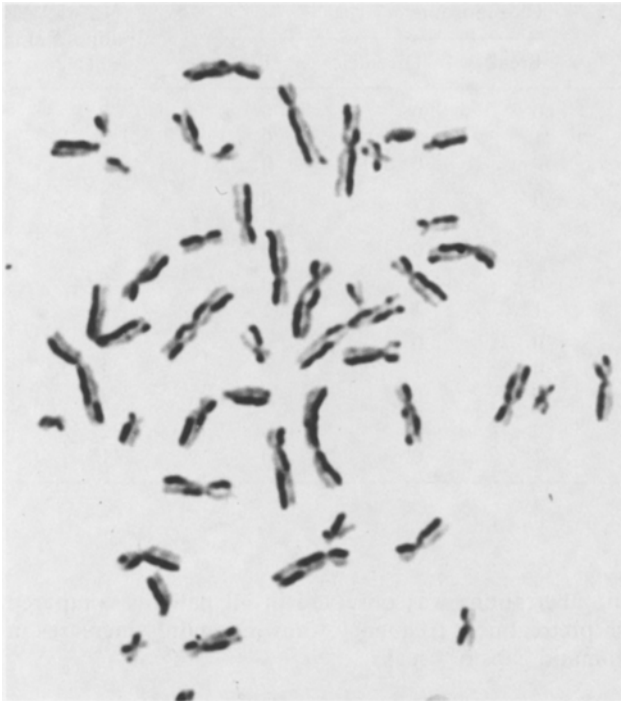

Fig. 2. Metaphase chromosome of lymphocyte from a patient (no. 1 in Table 3) 24 h after carboplatin treatment, showing multiple sister chromatid exchanges

in treatment. Bone marrow suppression by carboplatin is usually reversible in most patients receiving carboplatin, and they recovered to the pretreatment level about 3–4 weeks after treatment; this allows treatment with carboplatin to be repeated at 4–5 week intervals. It is interesting that clinical recovery of bone marrow suppression and recovery of SCE occurs within similar times, although only one case was tested for SCE in this study. It is yet unclear whether this decrease in SCE frequency is due to the repair of DNA damage or death of the damaged cells.

In agreement with a previous report demonstrating that cisplatin induced SCE more frequently than carboplatin in V79 cells in vitro [16], these results showed that cisplatin induced SCE more frequently than carboplatin at the same concentration. SCE frequency per chromosome in the presence of 0.05 μg/ml cisplatin was almost the same level observed in metaphases cultured with 1.0 μg/ml carboplatin, twenty times the concentration of cisplatin. On the other hand, the dose of carboplatin given to the patient was 4–5 times higher than that of cisplatin, and pharmacological studies have demonstrated that the average peak plasma concentration of carboplatin was about ten times higher than that of cisplatin (21.9 ±3.9 μg/ml and 2.49 ± 0.41 μg/ml, respectively) when 400–450 mg/m^2 carboplatin and 80–100 mg/m^2 cisplatin were given to the patients as single doses by i.v. injection over 15–60 min [6, 13]. Even considering the clinical dose of carboplatin or cisplatin, the ability of carboplatin to induce SCE may be lower than that of cisplatin.

Although most chemotherapeutic agents, particularly alkylating agents, that induce a significant level of SCE would be carcinogenic and the level of SCE may be related to the degree of carcinogenicity, the probability of the occurrence of second malignant neoplasms may largely depend on the viability of the cells affected by the cytotoxicity of the agents [1]. The mechanism of action of mitomycin C (MMC) is similar to that of classic alkylating agents, and MMC is a more potential SCE inducer than other potential carcinogens; however, second malignancies have been reported in only a few patients treated with MMC. An explanation for this may be that MMC is strongly cytotoxic, and DNA lesions caused by MMC are not long-lived [1]. Similarly, carboplatin is strongly cytotoxic against hematopoietic cells and induces myelosuppression, unlike cisplatin. DNA damage induced by carboplatin may be short-lived; therefore, it appears that carboplatin has less opportunity to be involved in the mutation process.

Chromosomal aberrations induced by carboplatin were mainly chromatid-type, such as gaps or breaks, in both in vitro and in vivo experiments; this type of chromosomal aberration has commonly been seen in cells exposed to chemical agents [4]. Previous study [11] has shown that cisplatin induces the dose-dependent increase of metaphases with chromosomal aberrations in vitro, and these effects were more dependent upon the concentration of cisplatin than upon the length of exposure. In human lymphocytes cultured with cisplatin at 0.5 μg/ml for 48 h, 56% of metaphases showed at least one break of exchange figure, and 14%, more than five breaks, with the mean number of breaks per cell being 3.0. It has also been demonstrated that the aberrations observed consisted primarily of chromatid breaks (gaps were not scored) [11]. In the present study, carboplatin significantly increased chromosomal aberrations, especially at 1.0 and 5.0 μg/ml; however, the ability of carboplatin to induce chromosomal aberrations may be lower than that of cisplatin, as in the results observed in SCE frequency. In fact, 14% and 18% of metaphases cultured with 5.0 μg/ml carboplatin showed chromatid breaks in the present study.

Experimental and clinical studies have demonstrated that the efficacy and spectrum of activity of carboplatin

are similar to those of cisplatin [3, 7, 18]. Although cisplatin has not be shown to be a carcinogenic in man, the present study suggests that carboplatin has an improved therapeutic index over the parent compound, cisplatin, because of its less mutagenic and/or carcinogenic hazard, in addition to the largely reduced incidence of untoward effects [3]such as nephrotoxicity, emesis, neurotoxicity, and ototoxicity.

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