[CANCER RESEARCH 53, 5669–5675, December 1, 1993]

Drug-induced DNA Modification in Buccal Cells of Cancer Patients Receiving Carboplatin and Cisplatin Combination Chemotherapy, as Determined by an Immunocytochemical Method: Interindividual Variation and Correlation with Disease Response[1]

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ABSTRACT

Twenty-six patients with a variety of tumor types were treated according to a phase 1 experimental treatment protocol consisting of repetitive cycles of *cis*-diammine(1,1-cyclobutanedicarboxylato)platinum(II) (carboplatin, 200–480 mg/m²) at day 1 and *cis*-diamminedichloroplatinum(II) (cisplatin, 50–100 mg/m²) at day 3. Buccal cells were collected in one or two treatment cycles prior to carboplatin, 24 h after carboplatin, just prior to cisplatin, and approximately 24 h after cisplatin administration. Drug-induced DNA modification was visualized at the single cell level by antiserum NKI-A59 and quantitated by microdensitometry.

All (39 of 39) treatments with carboplatin, and almost all (33 of 35) treatments with cisplatin resulted in an increase in nuclear stain. Interindividual variation in drug-induced, adduct-specific nuclear stain amounted to a factor of 5–8 for carboplatin and 5–12 for cisplatin. This drug-induced increase was, however, not related to the dose of either carboplatin or cisplatin, suggesting that large interindividual differences in DNA adduct formation and/or repair obscured the effects of dose variation within the relatively small range used for the drugs (2.4 for carboplatin and 2.0 for cisplatin). This explanation was strengthened by the good reproducibility of the immunocytochemical assay and by the reasonable correlation between carboplatin-induced nuclear stain in cycles 1 and 2 (correlation coefficient, 0.69; $P = 0.009$).

Mean carboplatin-induced nuclear stain was significantly higher in the first cycle than in the second cycle ($P = 0.0001$) but this difference was no longer significant when drug-induced nuclear stain was corrected for carboplatin dose. Differences in cisplatin-induced nuclear stain between cycle 1 and cycle 2 were small and not significant.

Carboplatin-induced nuclear stain was significantly higher in the partial responders than in the nonresponders ($P < 0.0001$, two cycles combined); the level of statistical significance remained the same after dose correction. Cisplatin-induced nuclear stain did not differ significantly between partial responders and nonresponders; this result might, however, be confounded to some extent by remaining carboplatin-induced nuclear stain at the moment of cisplatin administration.

It is concluded that determination of the extent of platinum-induced DNA modification might be helpful in predicting the tumor response in cancer patients.

INTRODUCTION

The interaction of platinum anti-tumor drugs such as cisplatin[3] and carboplatin with DNA is supposed to play an essential role in the cytotoxic effects of these drugs on tumor cells. The platinum-DNA adduct level in cultured cells and in animals often, but not always, correlates with cytotoxicity (1–3). For some cell lines the capacity to repair platinum-DNA adducts seems to be an important determinant of resistance against cisplatin or *cis*-dichloro(ethylenediamine)platinum-(II) (4, 5). It has also been suggested that the capacity of a cell to avoid programmed cell death (apoptosis) might play an important role in platinum resistance (6). A number of genes involved in apoptosis have been identified [*bcl-2,* (7); *myc,* (8); *p53,* (9)] and it has been reported that the resistance of Friend erythroleukemia cells to cisplatin correlates directly with c-*myc* expression (10).

The mechanisms of clinical resistance to cisplatin and carboplatin are not known. Investigations on the platination level of WBC DNA of patients with ovarian cancer or testicular cancer showed a correlation with disease response (11–13). A recent study by the same authors of a group of patients receiving carboplatin and cisplatin chemotherapy for a variety of tumor types also showed significantly higher adduct levels in responders as compared to nonresponders (14, 15). In these investigations on cancer patients DNA-bound platinum was analyzed by AAS and/or ELISA; a study in which both techniques were applied to the same series of human WBC DNA samples indicated, however, that a correlation between the results of the two assays on these samples was lacking (16).

In the above studies on cancer patients platination of WBC DNA was studied. For *in vivo* studies on the relation between DNA platination and tumor response in cancer patients, direct information about DNA platination in the tumor cells is to be preferred. This is, however, difficult because most tumors are not easily accessible. In addition, relatively large samples are needed for DNA analysis by ELISA and even more so for analysis by AAS. Furthermore, tumor heterogeneity will make it virtually impossible to draw conclusions about the DNA platination level in the tumor cells proper. The problems of sample size and tumor heterogeneity can, at least in principle, be solved by using immunocytochemical methods for the visualization and quantitation of platinum-DNA adducts.

We have developed immunocytochemical methods for the analysis of platinum and other DNA adducts at the cellular level (17, 18). Antiserum NKI-A59, raised against cisplatin-modified DNA (19), allows analysis of the interaction of cisplatin and carboplatin with DNA at the cellular level, also in the platinum-treated cancer patient (1, 20–22). In the present pilot study the possibility of immunocytochemical analysis of platinum-DNA adducts in an easily accessible cell type, the buccal cell, was further explored in a group of cancer patients treated with different dose combinations of carboplatin and cisplatin. It was expected that such combinations would allow administration of higher total doses of platinum compounds with still acceptable toxicity (22, 23). Clinical data on tolerance, toxicity, and tumor response in this group of patients, including some preliminary immunocytochemical data, have been published (22).

Received 5/20/93; accepted 9/27/93.

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[1] This study was supported by Grants NKI 86–11 and NKI 90–17 from the Dutch Cancer Society.

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[3] The abbreviations used are: cisplatin, *cis*-diamminedichloroplatinum(II); carboplatin, *cis*-diammine(1,1-cyclobutanedicarboxylato)platinum(II); AAS, atomic absorption spectroscopy; ELISA, enzyme-linked immunosorbent assay; PR, partial response; NR, no response; MR, minor response; PAP, peroxidase-(rabbit) antiperoxidase complex; GAR, goat anti-rabbit immunoglobulin; a.u., arbitrary units.

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MATERIALS AND METHODS

Treatment of Patients with Carboplatin and Cisplatin. Twenty-six cancer patients were treated at the Division of Medical Oncology of the Kenneth Norris Jr. Cancer Center, University of Southern California, on a phase 1 experimental treatment protocol (22). Carboplatin (200–480 mg/m^2) was administered on day 1 and cisplatin (50–100 mg/m^2) was gven on day 3 of 28-day treatment cycles (see Ref. 22 for details). Information on the patients (sex, age, and tumor type; number of cycles and doses of carboplatin and cisplatin used) is listed in Table 1. Three patients (patients 1, 9, and 16) had received prior cisplatin or carboplatin. As doses were escalated, only previously untreated patients were entered to avoid unpredictable myelosuppression. Dose modifications were not made during the first two cycles, with the single exception noted in Table 1. Carboplatin was dissolved in 5% dextrose in water and administered as a 30-min i.v. infusion. Cisplatin was dissolved in 0.9% NaCl in water and given as a 30-min i.v. infusion after prehydration of the patient with 0.9% NaCl in water. Clinical disease response is expressed as either PR, *i.e.*, a >50% reduction in tumor size lasting for more than 4 weeks, MR when the changes were slightly below 50% and lasting for more than 4 weeks, or NR when progression or lesser changes were recorded in serial tumor measurements. If not explicitly stated otherwise, the minor responders are combined with the nonresponders when the comparison PR *versus* NR is made.

Collection and Processing of Buccal Cells. Buccal cells were collected by repeatedly wiping the inner cheek with a cotton swab prior to carboplatin treatment (day 1; pre-carboplatin), 24 h after carboplatin (day 2; post-carboplatin), just prior to cisplatin (day 3; pre-cisplatin), and approximately 24 h after cisplatin (day 4; post-cisplatin). In some patients buccal cells were also collected on day 7. Cells were suspended in RPMI 1640 (Gibco, Paisley, United Kingdom), centrifuged for 10 min at 200 × *g*, and washed twice with phosphate-buffered saline. Cytospin preparations were made on ovalbumin-coated slides (20) and sent to the Netherlands Cancer Institute for immunocytochemical analysis of platinum drug-induced DNA modifications.

Immunocytochemical Analysis of Platinum-DNA Modification. The immunoperoxidase staining procedure was carried out as described (20), with omission of the RNase treatment since the latter did not influence the nuclear stain level. The general outline of the method was as follows. Cytospin slides were treated with phosphate-buffered saline-H_2O_2 (to inactivate endogenous peroxidases), 1 M KCl, proteinase K, ethanol-NaOH (the last 3 steps served to denature the DNA and to increase the accessibility of the platin-DNA adducts), 10% fetal calf serum (to reduce nonspecific antibody binding) and rabbit antiserum NKI-A59 against cisplatin-modified calf thymus DNA (19). Antibodies bound in the last step were visualized by triple PAP staining, *i.e.*, by sequential incubation of the cytospin preparations in GAR, PAP, GAR, PAP, GAR, and PAP; 3,3'-diaminobenzidine-HCl served as peroxidase substrate. Each sample was stained in duplicate in separate stainings on different days. A positive control, consisting of buccal cells from healthy persons incubated *in vitro* for 1 h at 37°C with 5 μg/ml cisplatin, was included in each staining to allow comparison of results from separate stainings.

The staining intensity of individual nuclei was measured as described previously (20). In each of two independently stained slides, the nuclear stain (defined as the sum of absorbances of the stained nuclear pixels; see Ref. 24) of 15–20 randomly selected nuclei was measured and expressed in a.u. To compare adduct levels in buccal cells of patients who had been treated with different doses of carboplatin or cisplatin, the nuclear stain was expressed per molar dose (a.u./mmol/m^2).

Data Analysis. Immunocytochemical analysis (including quantitation) was performed on 161 samples from 26 patients; the samples had been coded at the Kenneth Norris Jr. Cancer Center, Los Angeles. The code was broken at the end of the study. Increases in adduct levels were analyzed using unbalanced repeated measurement analysis of variance with unstructured covariance matrix (25). Computations were performed with the program 5V of the statistical computer package BMDP-PC90 (26). This method also provides correlation coefficients between several measurements within the same patient. Correlation coefficients between drug dose and nuclear stain, and also between nuclear stain in cycles 1 and 2 were calculated from linear regression analysis. Assumptions of analysis of variance (normality, constancy of variance) were graphically checked on the basis of residual plots and found to be reasonably satisfied.

RESULTS

Patients. Twenty-six patients were studied during one or more cycles of chemotherapy for platinum-DNA adducts in buccal cells. Table 1 gives information on these patients, including tumor type, drug dose, number of cycles, and disease response. Twenty different tumor types were observed; all 26 patients were evaluable for disease response. The major toxicity was thrombocytopenia (22). Partial responses were observed in patients with ovarian adenocarcinoma (patient 1), large cell lung cancer (patient 4), pleural mesothelioma (patient 7), and squamous cell carcinoma of the buccal mucosa (patient 11) and the esophagus (patient 14). One patient with a squamous cell carcinoma of the hypopharynx (patient 22) and one patient with a poorly differentiated carcinoma with unknown primary (patient 24) showed a 40% reduction in tumor size for more than 4 weeks; these two patients were classified as minor responders. In the above-mentioned patient with pleural mesothelioma, disease could not be objectively measured at the initiation of therapy. However, the patient experienced marked reduction in tumor bulk, complete resolution of malignant effusions, and marked improvement in clinical status as a result of therapy. We therefore included this patient in the group of partial responders for all analyses.

Platinum Drug-induced DNA Modifications in Buccal Cells. Nuclear stain values of buccal cells before and after treatment of the patients with carboplatin and cisplatin in the first and second cycles are depicted in Fig. 1. Complete data on 39 carboplatin infusions (23 first cycle; 16 second cycle; 13 patients with both first and second cycle) and 35 cisplatin infusions (19 first cycle; 16 second cycle; 11 patients both first and second cycle) were collected. Data from cycles with one or more pre- or posttreatment samples missing because patients had not shown up or because of the poor quality of a sample were rejected for further analysis.

Pre-carboplatin samples of patients who had ever received platin-based chemotherapy before the present study (patients 1, 9, and 16) did not show higher nuclear stain values than pre-carboplatin samples of patients who had never been treated with platinum drugs before. Pre-carboplatin nuclear stain values of the second cycle [6.2 ± 0.6 (SE) a.u.; $n = 13$] were slightly higher than but not significantly different ($P = 0.13$) from pre-carboplatin (= background) values of the first cycle (5.0 ± 0.4 a.u.; $n = 13$). Statistically significant increases in nuclear stain were observed after all treatments with carboplatin and after all but two treatments with cisplatin. Differences between pre- and post- values for either carboplatin or cisplatin were highly significant for all drug treatments, irrespective of the response, type of drug used, and the cycle ($P < 0.0001$).

In the first cycle the interpatient variation in adduct-specific nuclear stain (post-pre) was 6-fold for carboplatin and at least 12-fold for cisplatin. In the second cycle this variation was somewhat smaller, 5-fold for carboplatin, and 6-fold for cisplatin. This variation was larger than the range of dose levels, which amounted to a factor of only 2.4 for carboplatin and 2.0 for cisplatin. After correction for dose, interpatient variations in nuclear stain induced by carboplatin and cisplatin were 8-fold and at least 9-fold, respectively, in the first cycle and 5-fold for both drugs in the second cycle. No significant correlation between drug dose and drug-induced, adduct-specific nuclear stain was found (Fig. 2); correlation coefficients were 0.3 or lower. In the course of the experiments, the reproducibility of the staining protocol and the method for the quantitation of the nuclear stain was determined repeatedly. Typical results are shown in Fig. 3; the reproducibility proved to be satisfactory.

Differences in Adduct-specific Nuclear Stain in Relation to Drug, Cycle, and Response. Data on all 26 patients could be used to compare adduct-specific nuclear stain in buccal cells with disease response (patient numbers for each of the drug/cycle combinations are

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Table 1 *Information on evaluable patients including drug dose, tumor type, tumor response, and number of cycles*

Drug doses in cycle 1 and cycle 2 were identical with the exception of patient 16, who received 300 mg/m^2 carboplatin in cycle 1 and 225 mg/m^2 in cycle 2. Carbo, carboplatin; Cis, cisplatin. Earlier treatments: patient 1, cisplatin (9 months earlier), doxorubicin and cyclophosphamide; patient 6, cyclophosphamide, methotrexate, 5-fluorouracil, and doxorubicin; patient 7, doxorubicin and β-interferon; patients 8 and 10, 5-fluorouracil; patient 9, cisplatin (2 months earlier) and 5-fluorodeoxyuridine; patient 11, methotrexate; patient 16, carboplatin (2 months earlier) and vinblastine.

Patient number	Age	Sex	Regimen	Conc (mg/m2) CBDCA	Conc (mg/m2) cDDP	Tumor type	Response	Number of cycles anal	Legend
1	78	F	A	200	75	Ovarian, adenocarcinoma	pr	1	c1
2	52	M		200	75	Colon, adenocarcinomal	nr	1	c1
3	43	M		200	75	Parotid gland cancer (poorly diff)	nr	2	c1 c2
4	57	F		200	75	Lung, large cell cancer	pr	2	c1 c2
5	64	F		200	75	Parotid, adenoid cystic cancer	nr	2	c1 c2
6	27	F		200	75	Breast, infiltrating ductal	nr	1	c2
7	63	M	B	240	75	Pleural, mesothelioma	pr	2	c1 c2
8	60	M		240	75	Pancreas, adenocarcinoma	nr	1	c1
9	42	M	C	400	50	Unknown primary in liver	nr	1	c1
10	73	F		400	50	Pancreas, adenocarcinoma	nr	1	c1
11	52	M		400	50	Buccal mucosa, mucoepidermoid	pr	1	c1
12	45	M	D	480	50	Pancreas, adenocarcinoma	nr	2	c1 c2
13	84	M		480	50	Lung, large cell cancer	nr	1	c2
14	51	F		480	50	Esophagus, squamous cell cancer	pr	2	c1 c2
15	48	M	E	300	100	Adrenal, adenocarcinoma	nr	1	c1
16	65	F		300	100	Lung, poorly diff, superior sulcus cancer	nr	2	c1 c2
17	78	F		300	100	Lung, adenocarcinoma	nr	1	c2
18	62	M		300	100	Lung, squamous cell cancer	nr	2	c1 c2
19	45	F		300	100	Lung, squamous cell cancer	nr	2	c1 c2
20	54	F	F	480	75	Ovarian, adenocarcinoma	nr	2	c1 c2
21	40	F		480	75	Ovarian, adenocarcinoma	nr	2	c1 c2
22	60	M		480	75	Hypopharynx, squamous cell cancer	pr/mr	1	c1
23	66	F		480	75	Lung, adenocarcinoma (mod diff)	nr	2	c1 c2
24	59	F		480	75	Unknown prim, poorly diff cancer in axilla	pr/mr	2	c1 c2
25	54	M		480	75	Unknown prim, adenocarcinoma in chest wall	nr	1	c1
26	33	M		400	75	Nasopharynx, poorly diff cancer	nr	1	c1

included in Table 2). Table 2 also shows the levels of significance for the differences in adduct-specific nuclear stain for all 8 combinations of response, drug, and cycle. Since no clear correlation was observed for either carboplatin or cisplatin between drug dose and drug-induced (= adduct specific) nuclear stain, we decided to compare different groups and treatments both without and after correction of the adduct specific nuclear stain for the administered dose. Unless stated otherwise, results of the statistical analysis pertain to the comparison of PR *versus* the combined group of NR + MR.

Comparison without Dose Correction. Adduct-specific nuclear stain induced by carboplatin in the first cycle (partial responders and nonresponders combined) was significantly higher ($P = 0.0001$) than the corresponding value in the second cycle. Such a difference between the two cycles was not observed in the case of cisplatin ($P = 0.82$). Because of this difference in carboplatin-induced nuclear stain between cycles 1 and 2, the correlation between response and carboplatin-induced nuclear stain also had to be determined separately for cycles 1 and 2.

Some evidence was found for a second order interaction between drug and response; *i.e.*, the difference in drug-induced nuclear stain between partial responders and nonresponders was larger for carboplatin than for cisplatin ($P = 0.044$). Carboplatin-induced nuclear stain was significantly higher in the partial responders than in the nonresponders, both in the first ($P = 0.0001$) and in the second cycle ($P = 0.0048$). When the two cycles were combined, a P of 0.0001 was found. Although the mean nuclear stain after the first carboplatin infusion was a factor 2.0 higher in partial responders than in nonresponders, an overlap of about 25% for this parameter was found between the two groups. Linear regression analysis revealed a significant correlation between the carboplatin-induced nuclear stain in the first cycle and that of the same patient in the second cycle (Fig. 4; correlation coefficient, 0.69; $P = 0.0090$). No statistically significant difference in cisplatin-induced nuclear stain was observed between partial responders and nonresponders ($P = 0.33$); for this comparison data on the first and second cycle were taken together since there was no statistically significant difference between the two cycles. There was also no evidence for a correlation between the cisplatin-induced nuclear stain in the first and the second cycle (correlation coefficient, 0.08; $P = 0.81$). A comparison between drug-induced nuclear stain after carboplatin and the corresponding value of the same patient after cisplatin showed that there was no statistically significant correlation between these two entities (first cycle, correlation coefficient, 0.20; $P = 0.40$; second cycle, correlation coefficient, 0.25; $P = 0.36$).

Comparison after Dose Correction. Group differences between pre- and posttreatment values for carboplatin as well as cisplatin were highly significant for all drug treatments, irrespective of the response, type of drug used, and the cycle ($P < 0.0001$).

Differences in drug-induced nuclear stain between cycles 1 and 2 were again not significant for cisplatin ($P = 0.35$) and no longer significant for carboplatin ($P = 0.10$). Therefore, the data of both cycles were combined when the relation between drug-induced nuclear stain and tumor response was analyzed. The carboplatin-induced nuclear stain remained significantly higher in the partial responders than in the nonresponders ($P = 0.0001$). For cisplatin again no statistically significant difference was found between partial responders and nonresponders ($P = 0.80$). A weakly significant correlation was found to exist between the nuclear stain induced by carboplatin in the first cycle and that in the second cycle (correlation coefficient, 0.56; $P = 0.046$); such a correlation was again lacking for cisplatin (correlation coefficient, 0.08; $P = 0.81$). Finally, we compared the carboplatin-induced nuclear stain with the cisplatin-induced nuclear stain in the same patient but found no evidence for a correlation between these parameters (first cycle, $P = 0.16$; second cycle, $P = 0.09$; both cycles combined, $P = 0.41$).

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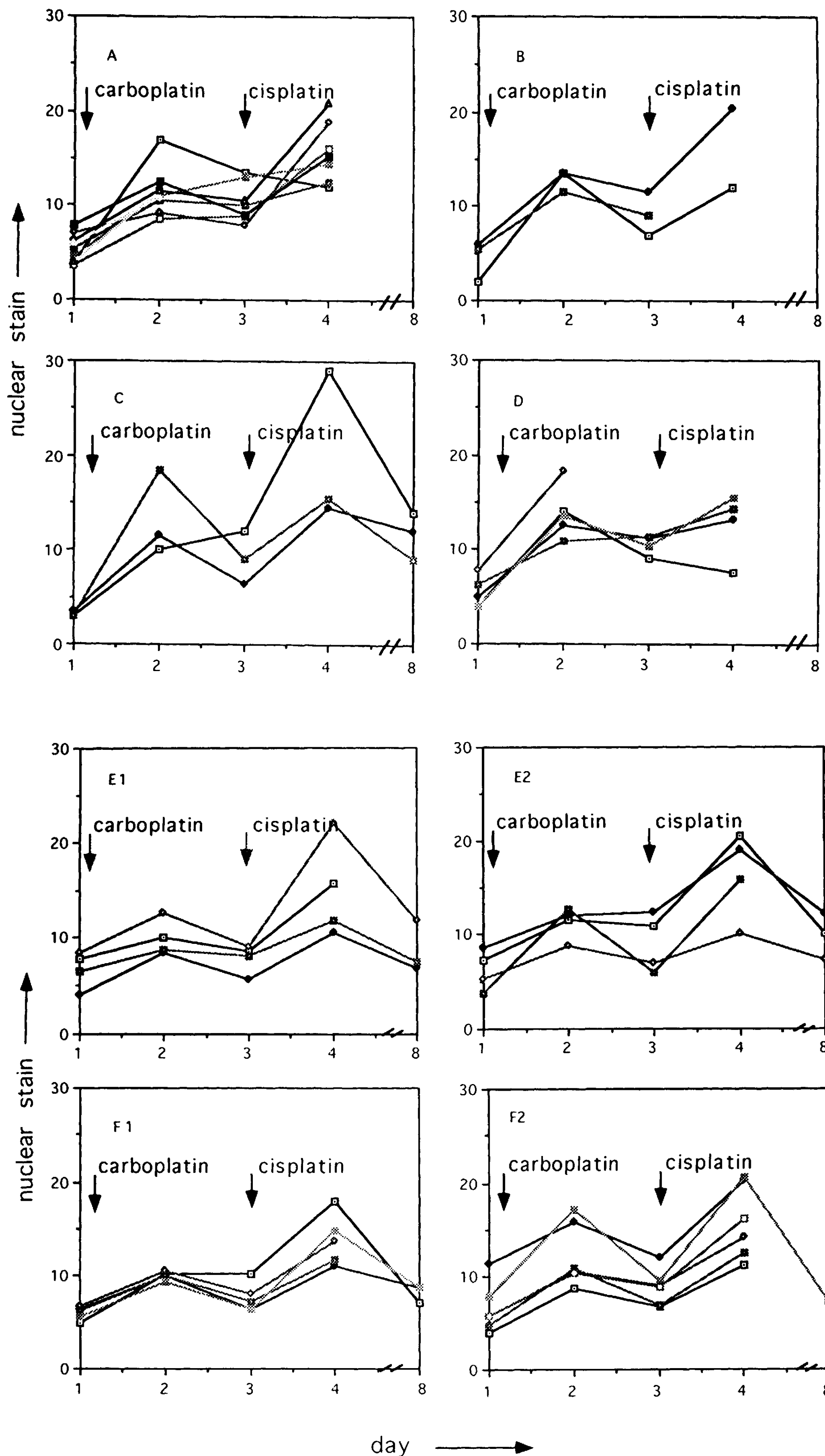

Fig. 1. Carboplatin- and cisplatin-induced DNA modification in buccal cells of patients treated with the drug regimens A-F specified in Table 1. *Arrows*, treatment with carboplatin (day 1) and cisplatin (day 3). Platinum drug-induced DNA modification was visualized immunocytochemically; the nuclear stain was quantitated and expressed in a.u. In case of treatment schemes E and F, the numbers of patients were too large to be included in a single panel. The symbols used to discriminate individual patients and cycles are explained in Table 1.

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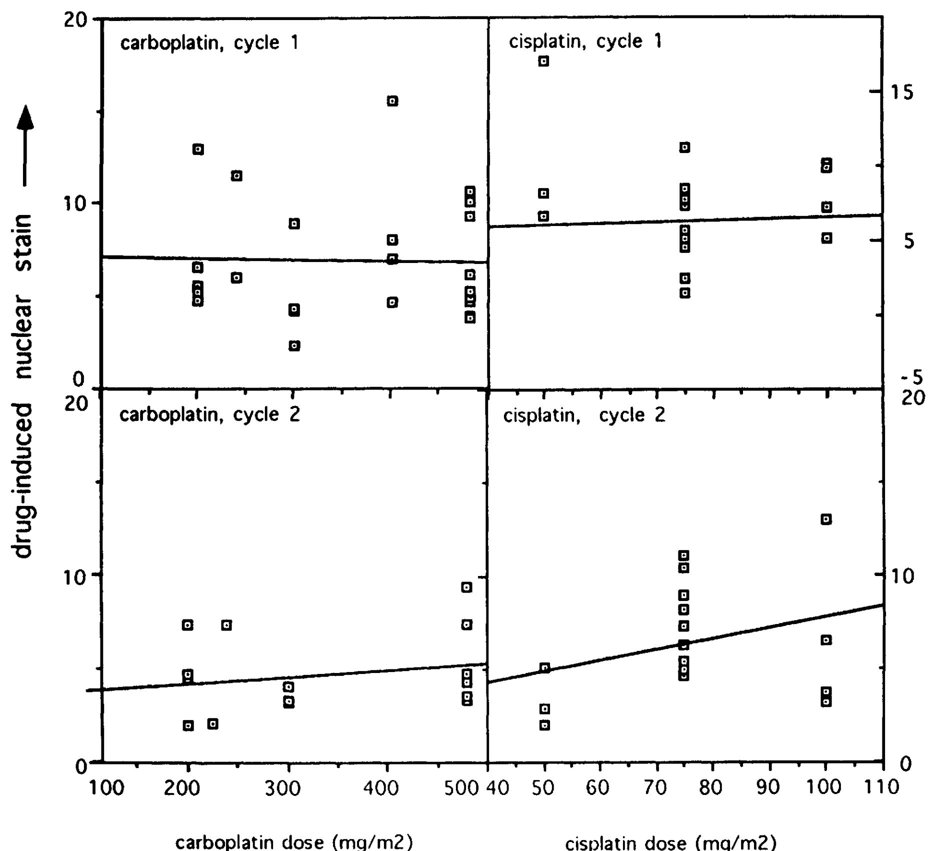

Fig. 2. Drug-induced nuclear stain (posttreatment minus pretreatment value) in buccal cells in relation to the dose of carboplatin and cisplatin. The lines were obtained by linear regression analysis; note that zero drug doses (pretreatment controls) are not included. The following correlation coefficients were calculated. Carboplatin: cycle 1, 0.03; cycle 2, 0.2. Cisplatin: cycle 1, 0.03; cycle 2, 0.31.

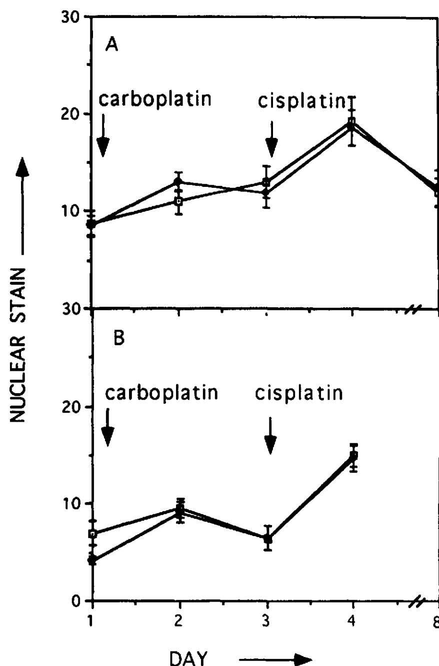

Fig. 3. Two identical series of buccal cell samples from patient 18, cycle 2 (*A*) and from patient 22, cycle 1 (*B*) were independently stained and quantitated. Nuclear stain (mean ± SE) is given in arbitrary units.

DISCUSSION

This pilot study was performed primarily to test the feasibility of immunocytochemical platinum-DNA adduct analysis in a group of cancer patients. The underlying assumption is that clinical resistance of human tumors to cisplatin and carboplatin is determined, at least to a substantial extent, by the level of drug-induced DNA modification and the persistence of the DNA adducts. Previous investigations on a small number of cancer patients had shown that an immunoperoxidase assay using antiserum NKI-A59 was suitable to visualize and quantitate both cisplatin- and carboplatin-induced DNA modification in buccal mucosa cells (20, 21). Microdensitometric quantitation of the adduct-specific nuclear stain does not yet yield absolute numbers of adducts per nucleus or per amount of DNA. Relative quantitation can, however, be achieved since almost linear relationships have been reported to exist between drug-induced nuclear stain and for instance drug dose, drug concentration, and DNA adduct level (for review, see Ref. 17).

Buccal mucosa cells were selected for the present study since they are easily accessible and can be sampled repeatedly on a single day. An advantage over WBC is that platin-DNA adduct-specific nuclear stain in our immunocytochemical assay is significantly higher in buccal cells than in leukocytes from the same patient. In a series of seven carboplatin-treated patients we found a reasonable correlation between DNA adduct-specific nuclear stain in buccal cells and WBC (correlation coefficient, 0.81, $P = 0.03$[4]).

The percentage of positive samples (98%) with a significant 24-h post-drug treatment DNA platination level is similar to that observed by Parker *et al.* (14) with AAS in WBC of cancer patients treated according to the same protocol, slightly above that observed by Fichtinger-Schepman *et al.* (27) with ELISA in WBC DNA of cisplatin-treated cancer patients but substantially higher than that observed by Poirier *et al.* (28) with ELISA in WBC DNA of cisplatin-treated cancer patients. These differences in percentages of positive samples may be related to dose rate of platin administration, loss of platin-DNA adducts due to repair and cell turnover, and/or the detection limits of the assays used (*cf.* Ref. 29).

The interindividual variation in adduct level is appreciable at a factor of 5–8 for carboplatin and 5–12 for cisplatin. An additional

[4] F. A. Blommaert *et al.*, unpublished observations.

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Table 2 *Drug-induced increase in nuclear stain in buccal cells of the patients listed in Table 1: analysis of possible relations between drug, cycle and response*

Drug-induced increase in nuclear stain is expressed in arbitrary units (mean ± SE) or, after correction for drug dose, in arbitrary units/mmol/m^2. Numbers in parentheses, number of patients. P cycle, comparison (for a single drug) of drug-induced increase in nuclear stain in cycle 1 with that in cycle 2; P response, comparison of drug-induced increse in nuclear stain between nonresponders (NR + MR) and partial responders (PR).

	Cycle 1	Cycle 2	Cycles 1 and 2	Cycle 1 *vs.* 2
Carboplatin, not corrected for drug dose				
N/M response	5.77 ± 0.52 (18)	4.00 ± 0.42 (13)		
Partial response	11.44 ± 1.02 (5)	7.98 ± 0.87 (3)		
P cycle				0.0001
P response	<0.0001	0.0048	<0.0001	
Cisplatin, not corrected for drug dose				
N/M response	6.36 ± 1.05 (15)	6.10 ± 0.82 (13)		
Partial response	2.05 ± 2.03 (4)	5.69 ± 1.70 (3)		
P cycle				0.82
P response			0.33	
Carboplatin, corrected for drug dose				
N/M response	5.65 ± 0.72 (18)	5.23 ± 0.76 (13)		
Partial response	13.72 ± 1.38 (5)	9.56 ± 1.57 (3)		
P cycle				0.10
P response			<0.0001	
Cisplatin, corrected for drug dose				
N/M response	23.70 ± 3.73 (15)	24.56 ± 2.82 (13)		
Partial response	15.86 ± 7.20 (4)	33.90 ± 5.86 (3)		
P cycle				0.35
P response			0.80	

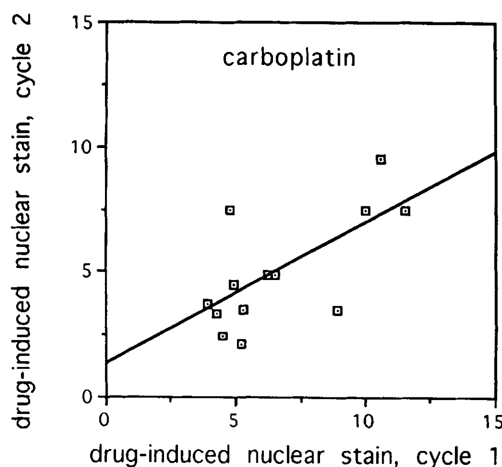

Fig. 4. Carboplatin-induced nuclear stain in cycle 1 *versus* cycle 2 is plotted for all patients (n = 13) who received two cycles with (for each individual patient the same dose of) carboplatin (correlation coefficient, 0.69; P = 0.009).

indication that this variation is really related to differences between individuals and not to methodological limitations of the immunocytochemical assay is the relatively good correlation between carboplatin-induced nuclear stain in the first cycle when compared with the second cycle. A statistically significant difference in drug-induced nuclear stain between the first and the second cycle was observed for carboplatin but not for cisplatin. It would be of interest to see if the lower carboplatin-induced nuclear stain in the second cycle is related to changes in the pharmacokinetics of this drug. Differences in pharmacokinetics and extent of drug-protein binding might also explain (part of) the large interindividual variation in drug-induced nuclear stain. This variation is held responsible for the lack of correlation between drug-induced nuclear stain and drug dose; additional factors are the small range of drug dose used in the present study and the relatively small numbers of patients. Hitherto unexplained extremely high adduct levels as incidentally observed for WBC DNA in a partially overlapping group of cisplatin- and carboplatin-treated (partially overlapping group) cancer patients by Parker *et al.* (14) were not encountered in the present, relatively large, group.

From the present experiments we conclude that quantitative immunocytochemical analysis of platinum-DNA adducts in serially sampled buccal cells of a relatively large group of cancer patients is feasible and gives reproducible results. Another encouraging conclusion regards the correlation between carboplatin-induced nuclear stain and tumor response.

It must, however, be emphasized that the patient population in this pilot study was a very heterogeneous one and that investigations on groups of patients with a single tumor type are needed to reach more definitive conclusions.

A strong correlation between drug-induced nuclear stain and disease reponse was observed for carboplatin in both cycles, both with and without dose correction. This correlation remained strong when the two minor responders were combined with the partial responders (PR + MR) and compared with the nonresponders (NR, without MR), although P values became a bit lower (range, 0.001–0.015). No other correlations between nuclear stain and disease response were found to exist: neither the decrease in nuclear stain between day 2 and day 3 (which might be considered an indication of the patient's capacity to repair carboplatin-induced DNA damage) nor the cisplatin-induced nuclear stain in any cycle could be related to disease response. The latter lack of correlation for the comparison PR *versus* NR + MR did not change when the minor responders were combined with the partial responders, *i.e.*, when the comparison PR + MR *versus* NR was made. This difference between carboplatin and cisplatin is remarkable since both drugs give rise to the same bifunctional adducts (30). It seems unlikely that the relatively small contribution of remaining carboplatin-induced DNA modification at the moment of pre-cisplatin sampling at day 3 obscured an otherwise good correlation between cisplatin-induced DNA modification and tumor response. The use of pre-carboplatin nuclear stain values as pretreatment values also for cisplatin did not improve the correlation with disease response. A practical point is that the time point selected for sampling (24 h after drug administration) proved to be optimal for carboplatin but later than optimal for cisplatin (18).

Our results are in line with those reported by Parker *et al.* (14) studying WBC from a partially overlapping patient population. These authors found a correlation for carboplatin, but not for cisplatin, between AAS-measured platinum-DNA adduct level and disease response. A recent update of this study (15) with more patients showed comparable results; a strong correlation with tumor response was found for first cycle carboplatin data and a much weaker one for first

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cycle cisplatin. These correlations became insignificant in the second cycle of treatment (15).

A drawback of all these studies (including the present one) is that drug-induced DNA modifications are determined in another cell type than the tumor cell. The observed correlations between tumor response and initial DNA platination in a surrogate cell type like the buccal cell or WBC suggest a correlation between DNA platination level in the tumor cell and that in the surrogate cell. The latter correlation might be due to the existence of genetically determined capacities for platinum drug excretion, transmembrane platinum transport, or intracellular scavenging of platinum drugs. Differences in drug excretion is expected to affect tumor cells and surrogate cells in the same way. The use of surrogate cell types in predicting tumor response might also be useful in case of small interindividual variation in the ratio of transport or scavenging capacities between the tumor cell and the surrogate cell. It remains difficult, however, to imagine that the initial DNA platination levels in buccal cells or WBC will quantitatively reflect this parameter in tumor cells when an originally platinum-sensitive tumor has become resistant to treatment. For this reason, future studies will have to concentrate on DNA platination in tumor cells. In this respect it is of interest that we[4] have been able to visualize *in situ*-induced cisplatin-induced DNA modifications in a human melanoma and several carcinomas of the uterine cervix.

ACKNOWLEDGMENTS

We thank Dr. A. M. J. Fichtinger-Schepman and Dr. F. Berends for critically reading the manuscript.

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