Cancer Chemother Pharmacol (1991) 27: 464–471
0344570491000400

Cancer Chemotherapy and Pharmacology

Influence of cisplatin on cell-cycle progression in xenografted human head and neck carcinomas

M. Jäckel and P. Köpf-Maier

Institut für Anatomie, Freie Universität Berlin, Königin-Luise-Straße 15, D-1000 Berlin 33, Federal Republic of Germany

Received 1 November 1989/Accepted 7 November 1990

Summary. The scope of the present study was to examine whether the cytokinetic phenomena occurring in human tumors under the influence of cisplatin correlate with the response of the tumors to therapy with the drug. Therefore, three strains of heterotransplanted human head and neck carcinomas showing different degrees of sensitivity to cisplatin were investigated by flow cytometry at various intervals after a single administration of cisplatin at four different dose levels (3, 6, 9 or 12 mg/kg). Three types of cell-cycle alterations were observed that depended on the dose of cisplatin and the degree of drug sensitivity shown by the tumors investigated. The obviously weakest kind of tumor reaction was a delay in the G_2 cell phase. This phenomenon also occurred in the case of non-responsiveness to therapy, whereby the growth, histological structure and mitotic activity of the tumors remained nearly unaltered after cisplatin treatment. With increasing cytotoxicity, additional accumulations of cells in the S phase and, finally, long-lasting blocks at the G_1/S boundary were found. The latter phenomenon, which manifested at high dose levels used in sensitive tumors, was obviously irreversible, as it did not completely disappear until the tumor cells had died and been removed by immigrating macrophages. It was always accompanied by severe histological destruction, tumor cell necrotization, and marked depression of the mitotic index. Thus, the hindrance of cell traversal through the S phase obviously represents the main and significant cytokinetic event, which indicates a potent antitumor effect for cisplatin that leads to pronounced tumor regression. This finding supports the hypothesis that inhibition of DNA synthesis is the mechanism underlying the cytotoxicity of cisplatin.

Introduction

cis-Diamminedichloroplatinum(II) (cisplatin) [23] is one of the drugs most widely used in current clinical trials of cytostatic agents. It is extremely effective in the treatment of testicular cancer, which is curable even in its advanced stages by cisplatin-based chemotherapy [14]. Further important indications for the use of cisplatin are ovarian, bladder, prostatic, uterine, and lung tumors as well as squamous-cell carcinomas of the head and neck [16]. However, as compared with response rates observed in patients with testicular cancer, those achieved in other tumors are considerably lower, with cures occurring only rarely. In head and neck carcinomas, the clinical use of cisplatin as a single agent induces partial and complete remissions in 26%–41% of all patients treated. Its combination with bleomycin, 5-fluorouracil and/or methotrexate enhances the response rate to 33%–76% [3, 9, 10, 12, 20, 26].

At the cellular level, disturbances of cell-cycle progression are observed during the administration of most antitumor agents [1]. Analysis of the cytokinetic effects is important, as it can improve the concepts involved in tumor treatment by giving a better basis for the scheduling and monitoring of cancer chemotherapy. Previous studies performed in vivo and in vitro using cisplatin in animal and human tumors revealed G_2 blocks following transient accumulations of cells in the S phase after drug administration [2, 15, 28]. In the continuation of these investigations, the scope of the present study was to examine whether there is a correlation between the observed cytokinetic events and the therapeutic efficacy of cisplatin. Thus, three human head and neck carcinoma strains were used that had been heterotransplanted into athymic mice and showed differing degrees of sensitivity to cisplatin. Their cytokinetic behavior, correlated with the histological alterations, mitotic activity and growth behavior of the tumors, was analyzed at four different cisplatin doses and at various intervals following each single-dose application.

Offprint requests to: P. Köpf-Maier

Materials and methods

Animals. Congenitally athymic male mice (NMRI, nu/nu) were kept in autoclaved cages set in laminar flow racks. Food and acidified water (pH 2.5) were made available ad libitum. At the beginning of the experiments, the animals were 6–8 weeks old and weighed about 18–22 g.

Tumors. In the present study we used two squamous-cell carcinomas derived from the head and neck of human patients. They were established in nude mice as serially transplanted tumors and showed different degrees of sensitivity to cisplatin. Whereas one tumor, a maxillary sinus carcinoma, responded only marginally to cisplatin, the other, a hypopharynx carcinoma, was highly sensitive to the drug. Independently from the initial difference in the responsiveness of the two tumors to cisplatin, partial drug resistance was experimentally induced in a strain derived from the highly sensitive hypopharynx carcinoma by the weekly administration of cisplatin doses increasing up to 3 mg/kg once a week. Thus, three tumor lines showing different degrees of sensitivity to cisplatin were available. By the time we began the present experiments, these tumor lines had undergone 7, 12 and 13 passages, respectively, through nude mice.

For transplantation purposes, the tumors were removed from donor animals, minced with scissors, pressed through injection needles and suspended in equal volumes of Hank's salt solution. A 0.4-ml volume of tumor suspension was inoculated subcutaneously into the right flank of mice. Around day 30 after tumor transplantation, when the tumors showed maximal growth, cisplatin was injected. The day on which cisplatin was given was defined as day 0 of the experiments.

Chemotherapy. Cisplatin was dissolved in a mixture of saline and dimethylsulfoxide (DMSO; 9/1, v/v) and immediately injected intraperitoneally in the mice. It is known that within 40 min after the dissolution of cisplatin in DMSO, the drug molecules remain intact and do not interact with the DMSO [13]. Groups of 20 tumor-bearing animals were treated with sublethal doses of 3, 6, 9 or 12 mg/kg cisplatin, respectively. After intervals of 12, 24, 36 and 48 h and 3, 4, 5, 6, 8 and 10 days, the tumors in two animals each were removed and frozen in fluid nitrogen. During the time course of these therapeutic trials, no death due to cisplatin toxicity was observed. This sublethal character of the doses used had been confirmed by numerous chemotherapy studies performed during recent years in our laboratory using the same mouse strain.

In all three experimental groups, three additional mice received the DMSO/saline mixture (1/9, v/v) without drug, the tumors in these animals serving as control lesions. Control and treated tumors were removed simultaneously at 24 h and at 5 and 10 days following cisplatin administration and were subjected to the same procedures. Comparative studies with totally untreated control tumors revealed that neither DMSO nor saline influenced the cytokinetic or histological properties of the tumors in any way.

Flow cytometry. Following defrosting, the tumors were minced with scissors and treated with a pepsin-HCl solution (0.2% HCl; 3,500 IU pepsin/l) for 8 min at 37° C. During this period of exposure, the tumor suspension was continuously pressed through injection needles and then filtered once through surgical gauze. After pepsination, the cell suspensions were centrifuged at 1,300 rpm for 7–8 min and resuspended in 70% ethanol, which rendered the samples storable at 4° C for several weeks. After additional centrifugation, the pellets were resuspended in a solution containing 10 mg ethidium bromide/l and 10 mM $MgCl_2$/l in 0.1 M TRIS buffer (pH 7.5) in such a manner that final concentrations of $1-2 \times 10^6$ cells/ml were attained. To eliminate unspecific staining reactions due to RNA, the cell suspensions were incubated for 30 min at 37° C with ribonuclease (Serva 34390), which was added at a final concentration of 10 µg/ml.

Flow-cytometric measurements were performed using an Epics 752 cytometer equipped with an argon ion laser (wavelength, 488 nm; laser power, 250 mW). The fluorescent light signals emitted by cell nuclei stained with ethidium bromide could be transformed into voltage impulses by a linear or logarithmic mode. For graphic presentation the latter was chosen, because the pattern of cell populations with higher nuclear DNA content was then more distinctly recognizable. Thus, the abscissa of the histograms showing the DNA content of the cell nuclei was scaled logarithmically for graphic purposes. For calibration of the abscissa, human lymphocytes isolated by a Percoll separation method were used as a DNA standard. To differentiate human tumor from murine stroma cells in the histograms, the DNA content of mouse and tumor nuclei was estimated by chromosome analysis and set in relation to that of the human lymphocytes. Because of the pronounced hyperploidy of the tumor strains investigated (70–80 chromosomes), the DNA content of human tumor and murine stroma cells (40 chromosomes) differed markedly, resulting in a clear separation of the murine cell peak and the G_1 population of the tumor cells. Totals of 15,000 cells were measured for each histogram. For determination of the coefficients of variation of the diverse peaks in the DNA histograms, the abscissa of the latter was scaled linearly. The obtained values ranged between 1.9% and 4.5%.

Light microscopy. Small pieces of each tumor were fixed for 2 days in Karnofsky's solution containing 3.75% glutaraldehyde and 3% paraformaldehyde in cacodylate buffer (pH 7.4). After postfixation in an aqueous solution of 1% osmium tetroxide for 1 h, they were dehydrated and embedded in Epon. Sections cut at a thickness of 0.75 µm were stained with toluidine blue. For determination of the mitotic indices, the number of mitoses in 2,000 cells/sample were counted and given in ‰.

Measurement of tumor growth. To examine the influence of cisplatin on tumor growth development, we randomized tumor-bearing animals into control and treatment groups consisting of 4–5 animals each. On day 10 after transplantation, by which time the tumors had reached a size of 0.3–0.6 cm^3, the treatment groups received 3, 6, 9, or 12 mg/kg cisplatin according to the manner described above. Every 7 days, two perpendicular diameters (length a, breadth b) of the tumors were measured with graduated calipers. Absolute tumor volumes were calculated using the formula $v = a \times b^2/2$. Relative tumor volumes were determined by relating the absolute values measured at 1 week or several weeks after treatment to that found on the day of drug injection. Mean values for treated and control animals were calculated for each experimental day. Then, T/C (treatment/control) ratios were attained using the formula:

$$T/C = \frac{\text{Mean relative volume of treated tumors}}{\text{Mean relative volume of control tumors}} \times 100\ (\%).$$

Results

Tumor growth behavior

Table 1 demonstrates the influence of a single dose of 9 mg/kg cisplatin on the growth behavior of the three head and neck carcinomas used in the present study. In the maxillary sinus carcinoma, the volume of the treated tumors diminished by 50% as related to that measured on

Table 1. T/C values determined at 1 or several weeks after the administration of 9 mg/kg cisplatin to nude mice bearing xenografted human tumors

Tumor	T/C (%)				
	7 days	14 days	21 days	28 days	35 days
MS	35	19	14	13	19
H-r	10	1	8	17	26
H-s	11	2	0	1	1

MS, Maxillary sinus carcinoma; H-r, hypopharynx carcinoma (partially resistant strain); H-s, hypopharynx carcinoma (original sensitive strain)

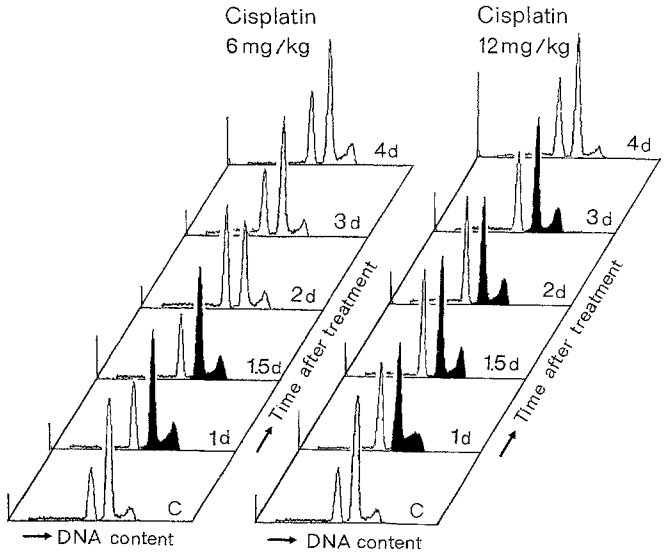


Fig. 1. DNA distribution curves for the maxillary sinus carcinoma at increasing intervals after treatment with 6 (*left*) or 12 (*right*) mg/kg, cisplatin. *Histograms marked with C* represent untreated control tumors; *black-shaded histograms* indicate peculiar features

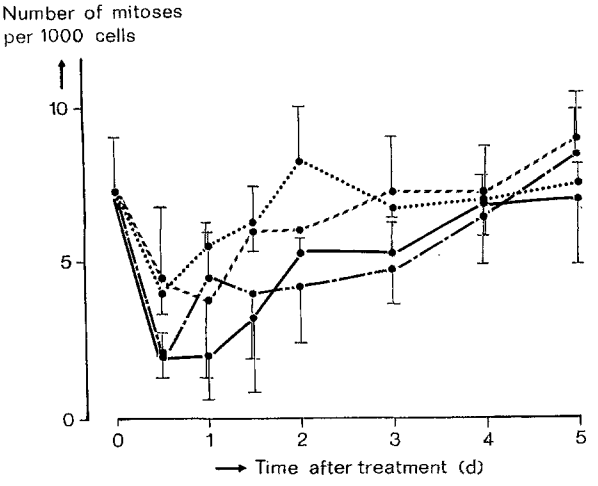


Fig. 2. Mitotic indices found for the heterotransplanted human maxillary sinus carcinoma at various intervals after the administration of 3 (.....), 6 (– – – –), 9 (–·–·–) and 12 (———) mg/kg cisplatin; 2,000 cells were evaluated per animal. The values for control tumors are given as day 0 values

the day of treatment, resulting in minimal T/C ratios of about 15% on days 21 and 28 after cisplatin administration. In both of the hypopharynx carcinoma strains, all tumors shrank by about 90% (to $\leq 10\%$ of their starting volumes) within 14 days, the T/C ratios then amounting to only 1%–2%. Whereas the resistant strain of this tumor recovered during subsequent weeks, no regrowth of tumors of the original sensitive strain was detectable. At the other cisplatin doses (3, 6 and 12 mg/kg), the growth-inhibiting effects were clearly dose-dependent, being either weaker (3 and 6 mg/kg) or intensified (12 mg/kg) as compared with the results obtained at the 9 mg/kg dose.

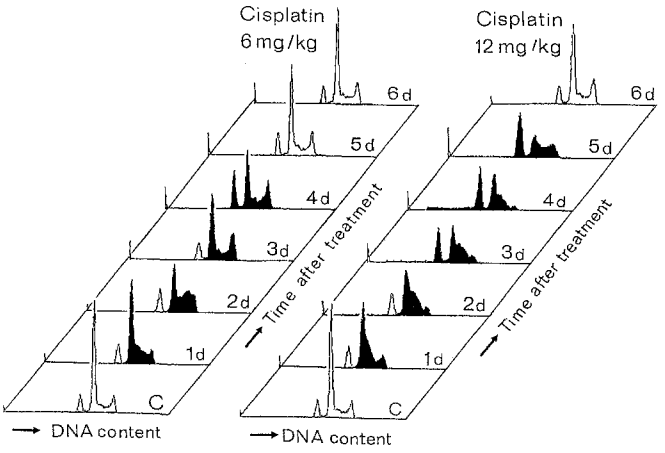


Fig. 3. DNA distribution curves for the partially resistant strain of a hypopharynx carcinoma at increasing intervals after treatment with 6 and 12 mg/kg cisplatin (for further explanation, cf. legend to Fig. 1)

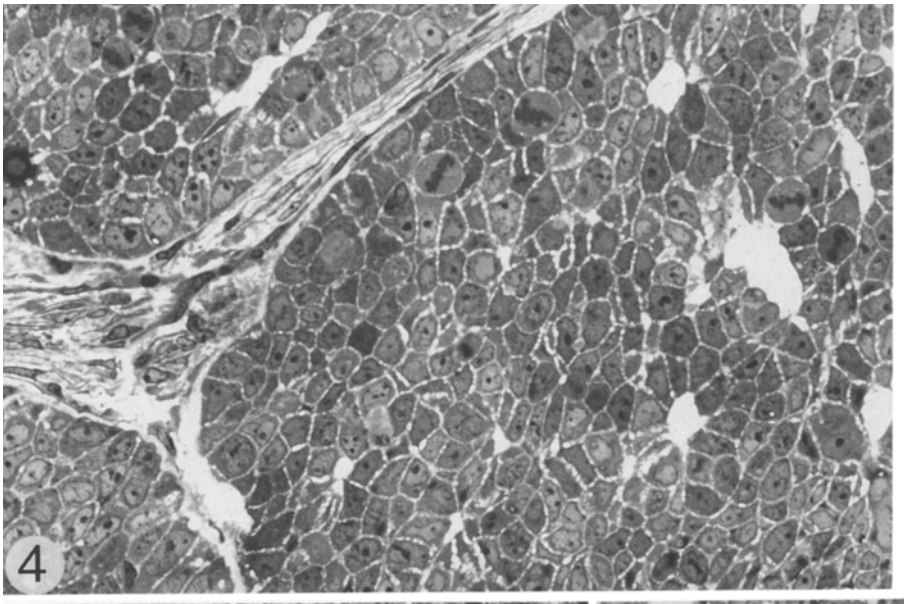
Maxillary sinus carcinoma

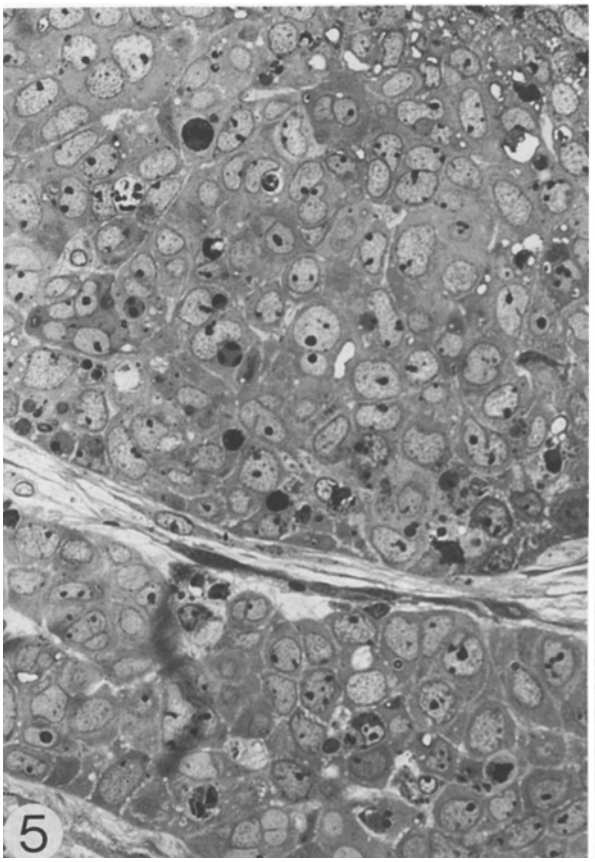
Figure 1 shows the cell-cycle alterations observed in the maxillary sinus carcinoma after therapy with 6 or 12 mg/kg. At the bottom of each column, the histogram of a control tumor (C) treated 24 h previously with the DMSO/saline mixture is shown. It comprises diverse peaks representing cell populations with different DNA contents. The area under each peak is proportional to the size of the respective population in the heterotransplant. The first peak on the left derives from mouse cells that always build up the stroma in heterotransplanted human tumors [27] and represented about 25%–30% of all cells in the maxillary sinus carcinoma xenograft. The other two peaks correspond to populations of tumor cells in the G_1 and G_2 phase, respectively; between these peaks, cells were found that were passing through the S phase of the cell cycle.

The histograms obtained after treatment with 6 mg/kg cisplatin revealed that a slight G_2 delay arose between 24 and 36 h after drug administration, after which the cytokinetic pattern again normalized. After a single dose of 12 mg/kg cisplatin, a longer-lasting G_2 arrest and, on day 1, the accumulation of a small number of cells in the late S phase were observable. Parallel to these cytokinetic events, no changes in the histological appearance of treated maxillary sinus carcinomas were detectable. The mitotic activity, illustrated in Fig. 2, was nearly unaffected by cisplatin doses of 3 and 6 mg/kg, whereas doses of 9 and 12 mg/kg resulted in slight, short-lasting and non-significant diminutions of the number of mitoses (significance level, $P > 0.01$).

Hypopharynx carcinoma

Partially resistant strain. Figure 3 documents cell-cycle alterations in the partially resistant strain of the hypopharynx carcinoma following treatment with cisplatin. In the untreated stage, the xenograft comprised about 10% murine stroma cells and 90% human tumor cells in diverse cell-cycle phases. The administration of 6 mg/kg cisplatin





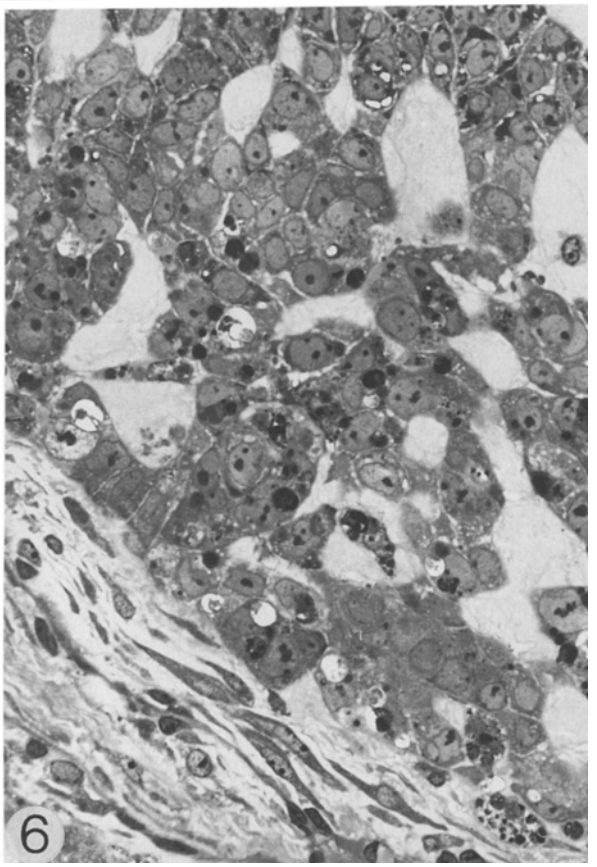


Fig. 4. Untreated human hypopharynx carcinoma xenografted to athymic mice. Semithick section, × 360.

Fig. 5. Resistant strain of the hypopharynx carcinoma at 2 days after the administration of 6 mg/kg cisplatin, showing single degenerated tumor cells containing cytoplasmic lipid droplets and nuclei with clumped chromatin among structurally unaltered tumor cells. Semithick section, ×360.

Fig. 6. Resistant strain of the hypopharynx carcinoma at 3 days after the administration of 12 mg/kg cisplatin, showing numerous tumor cells with irregularly shaped cytoplasmic inclusion bodies and the presence of inflammatory cells such as granulocytes and macrophages. Semithick section, ×360

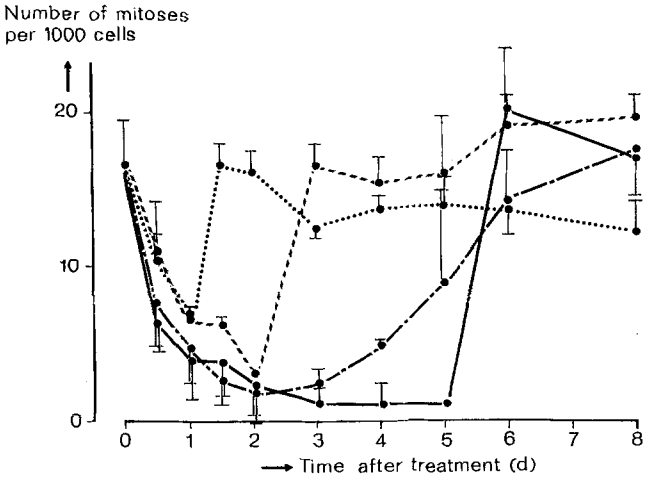


Fig. 7. Mitotic indices found for the partially resistant strain of a heterotransplanted human hypopharynx carcinoma after the administration of cisplatin as shown in Fig. 2

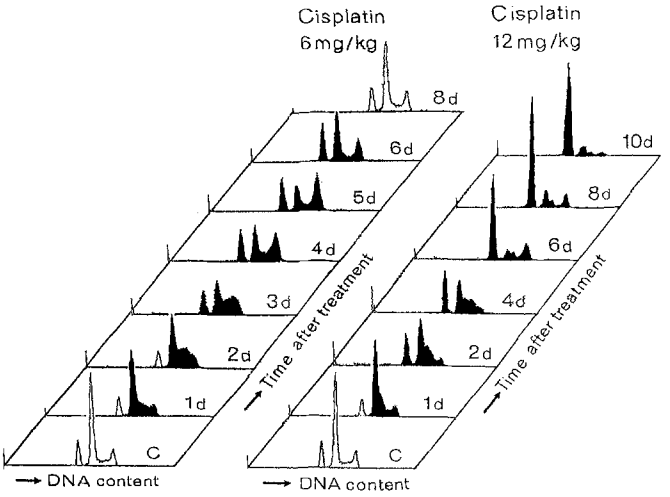


Fig. 8. DNA distribution curves for the hypopharynx carcinoma (original sensitive strain) at increasing intervals after treatment with 6 and 12 mg/kg cisplatin (for further explanation, cf. legend to Fig. 1)

effected a moderate delay of the S phase in tumor cells on days 1 and 2 after drug administration and an increase in the G_2 peak on days 3 and 4. At the high dose level (12 mg/kg), a pronounced accumulation of cells occurred at the G_1/S boundary within 24 h and persisted for 4 days. Apparently, these cells were blocked at the end of the G_1 phase and their synthesis of DNA was hindered. On day 5, this phenomenon seemed to be disappearing and a slight G_2 delay arose, which led to normalization of the histograms on day 6 after treatment. Parallel to these events, a moderate augmentation of host animal cells, represented by the first peak in the histograms, was observable around days 3–5.

Histologically, typical signs of cytotoxic damage were detectable after treatment with 6, 9 and 12 mg/kg cisplatin. Figure 4 illustrates the morphology of an untreated control tumor of the partially resistant strain of hypopharynx carcinoma. It represents a moderately differentiated squamous-cell carcinoma, with the tumor-cell clusters imitating the structure of the spinocellular stratum of the epidermis. No indications of ongoing necroses, bleeding or infiltrations of inflammatory cells could be detected in untreated tumors. At 2 days after the administration of 6 mg/kg (Fig. 5), a few degenerate tumor cells containing cytoplasmic lipid droplets, phagolysosomes and nuclei with clumped chromatin were observable. Treatment with 12 mg/kg cisplatin resulted in profound morphological alterations (Fig. 6). The typical tumor structure was disordered, whereby many tumor cells contained irregularly shaped cytoplasmic inclusion bodies reminiscent of phagosomes, cytolysosomes or residual bodies. Furthermore, some inflammatory cells had immigrated and obviously caused the increase seen in the mouse-cell peak in the histograms around day 4. Parallel to these phenomena, a significant ($P < 0.005$) depression of the mitotic activity manifested, which varied in duration with the cisplatin dose used (Fig. 7). It persisted for approximately as long as delays at the G_1/S boundary and in the S phase were detectable in the histograms of tumor cells.

Original sensitive strain. The cell-cycle perturbations observed after the administration of cisplatin to this tumor strain were considerably more pronounced and persisted for a longer period (Fig. 8) than those seen in the other, less sensitive tumor strains. During the first few days after treatment with 6 mg/kg cisplatin, accumulations of cells in the S phase and, some days later, in the G_2 phase were the main cytokinetic findings. By 8 days after drug treatment, the histograms had again normalized. In contrast to these findings, treatment with the high dose of cisplatin (12 mg/kg) resulted in the development of a marked and long-lasting G_1/S block, which appeared immediately after drug administration and was completed and partially replaced by a small G_2 block on day 6. At 8 and 10 days after treatment, at which times the G_1/S and G_2 blocks were present, the tumors were macroscopically observed to have practically disappeared. Parallel to this nearly complete tumor regression, the relative number of tumor cells in the histograms diminished, whereas the mouse-cell peak increased markedly and became the predominant cell population on days 6, 8 and 10.

The histological appearance of the untreated original sensitive hypopharynx carcinoma was identical to that of the partially resistant strain (cf. Fig. 4). Following the administration of 6 mg/kg cisplatin, signs of the ongoing necrosis of tumor cells and an invasion of inflammatory cells were the main morphological findings (Fig. 9). Treatment with 12 mg/kg completely destroyed the architecture of the tumor tissue within 6 days. During this period, most tumor cells degenerated, died and were phagocytosed and removed by immigrating host-supplied inflammatory cells (Fig. 10). In correspondence with the histological alterations observed at all cisplatin doses used, the mitotic activity of the sensitive hypopharynx carcinoma was diminished highly significantly ($P < 0.0005$), with nearly complete cessation of mitosis occurring after doses of 9 and 12 mg/kg (Fig. 11).

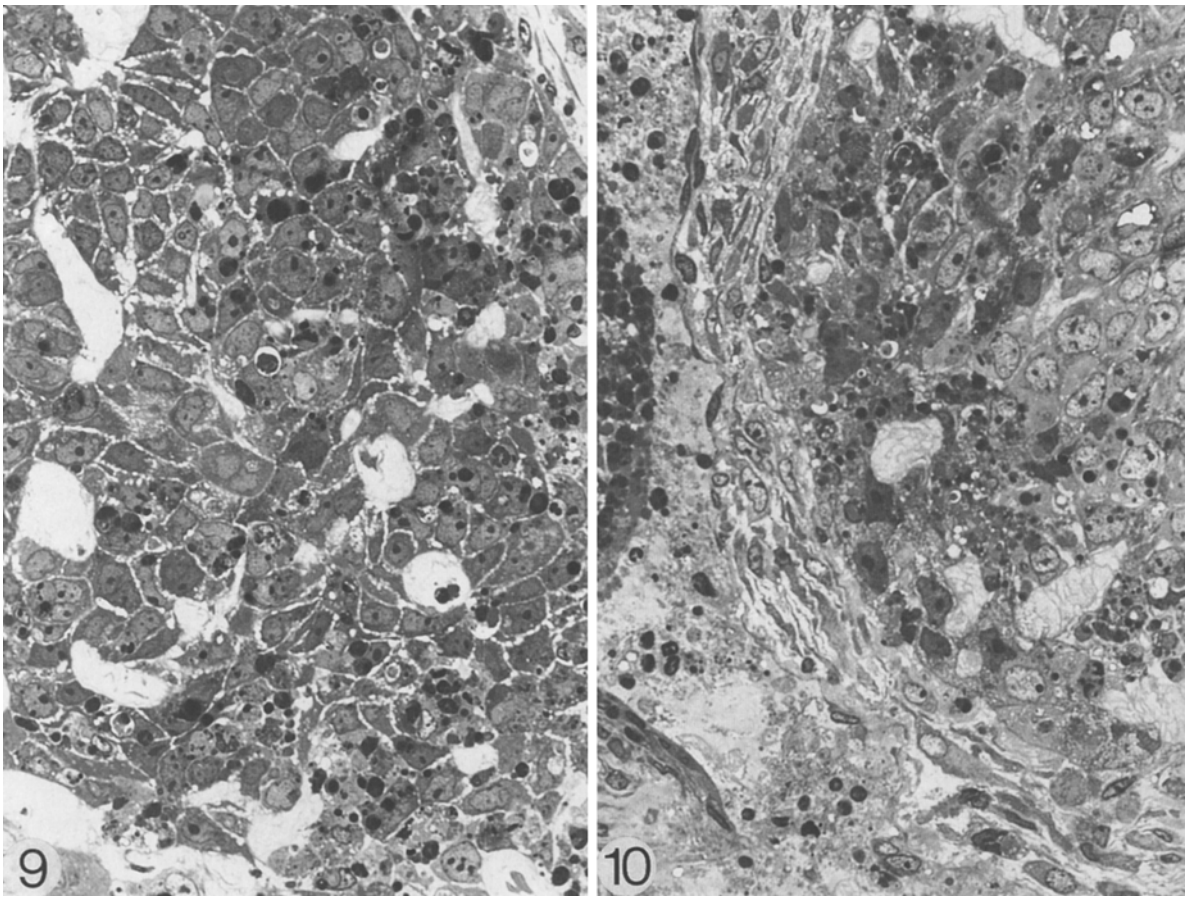


Fig. 9. Original sensitive strain of the hypopharynx carcinoma at 3 days after the administration of 6 mg/kg cisplatin, showing ongoing necrosis of many tumor cells and immigration of inflammatory cells. Semithick section, × 360.

Fig. 10. Original sensitive strain of the hypopharynx carcinoma at 4 days after the administration of 12 mg/kg cisplatin, showing destruction of tumor tissue and phagocytosis of injured tumor cells by host-supplied inflammatory cells. Semithick section, ×360

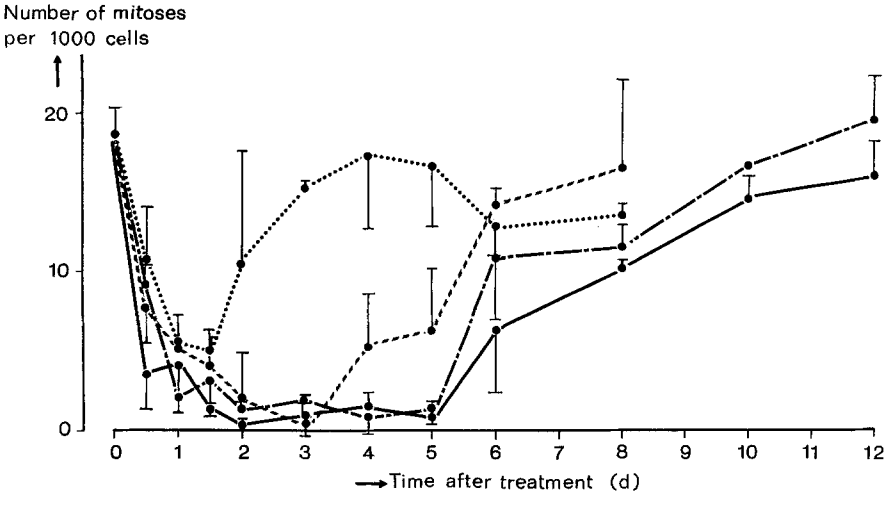


Fig. 11. Mitotic indices found for the heterotransplanted human hypopharynx carcinoma (original strain) after the administration of cisplatin as shown in Fig. 2

Discussion

In the present study, the influence of the antineoplastic drug cisplatin on the cytokinetic behavior of three heterotransplanted human head and neck carcinomas was analyzed according to the dose of drug and the degree of tumor responsiveness to cisplatin therapy. The following conclusions can be drawn from the results. The G_2 block, which was induced either by low doses of cisplatin in sensitive tumors or by high doses in resistant strains, apparently represents a weak and unspecific cytokinetic phenomenon that is not accompanied by extensive destruction of tumor tissue and tumor regression. On the other hand, delays occurring in the S phase or at the G_1/S boundary obviously confirm the drug's pronounced cytotoxic effect, which is also reflected by severe histological alterations, especially the necrotization of numerous tumor cells, a marked depression of mitotic activity and a significant diminution of tumor volume. The duration and magnitude of this G_1/S block seem to indicate the strength of the cytotoxic action. The G_1/S block probably results from inhibition of DNA synthesis, which is generally supposed to be the crucial effect caused by the interaction of cisplatin with DNA [18, 22, 29]. Studies using DNA electrophoresis have shown that DNA synthesis is blocked at nucleotides at which platinum binds to DNA molecules [19]. Moreover, Harder et al. [7] demonstrated a reduced affinity of human DNA polymerases for a platinated template and could show that the observed inhibition of DNA synthesis was unlikely to be the consequence of platinum linkage to enzymes. This result has been supported by other experiments, which confirmed that DNA is the most important macromolecular target of intracellular platinum binding [17]. Thus, a direct attack by cisplatin on DNA actually seems to be the action that is most probably responsible for the cytotoxic effect of the drug and, hence, for the blocked or delayed cell traverse through the DNA-synthesizing phase represented by G_1/S or S blocks in the DNA histograms.

In terms of the G_2 arrests that occurred during the dissolution phase of reversible G_1/S and S blocks as well as after treatment of non-responding tumors, other authors suppose that the inability of the cells to produce transcripts needed for mitosis during the G_2 phase [24, 25] might be responsible for the induction of G_2 blocks. Although it is known that transcription and protein synthesis are required for the passage of cells into mitosis [21], it is difficult to understand why transcription reactions in G_2 would be inhibited, whereas DNA synthesis would not yet be affected. These doubts have been confirmed by previous in vivo and in vitro studies showing that impairments of RNA and protein syntheses simply represent additional and concomitant effects at high doses of cisplatin [6, 11]. Thus, it is conceivable that the cell-cycle perturbations caused by an inhibition of DNA synthesis, such as G_1/S and S blocks, are followed by G_2 delays, but the G_2 delays that occur in less sensitive tumors without preceding G_1/S or S blocks require another mechanism. Conformational changes in platinated DNA, which manifest on [^{1}H]-NMR spectrometry as a loss of normal stacking interactions in the trinucleotide d(GpCpG) after platination [8], could be one of the reasons leading, as consequence of the distorted geometry of DNA helices, to a delay in chromosome condensation, which usually begins at the end of the G_2 phase. On the other hand, it seems probable that the different extent of cell arrest in G_2 observed in the tumors investigated in the present study could depend on the velocity and effectiveness of DNA repair processes, which are supposed to be important determinants of cisplatin sensitivity [4].

Cisplatin-induced cell-cycle alterations have previously been described in several studies. Köpf-Maier et al. [15] incubated Ehrlich ascites tumor cells in vitro with different doses of cisplatin and found G_2 blocks following retardations of cell transition through the S phase. At the highest, cytotoxic concentrations, the cells were immediately and irreversibly arrested in the cell cycle and died. Wennerberg et al. [28] reported the behavior of a heterotransplanted squamous-cell carcinoma of the nasal cavity following the administration of cisplatin. These authors showed moderate cell accumulation at the G_1/S boundary and, thereafter, a delay in cells passing through the G_2 phase. Human colon-cancer cells investigated in vitro by Bergerat et al. [2] were slightly arrested in G_2 after exposure to low concentrations of cisplatin. Increases in the drug dose and the exposure period resulted in cell-cycle blocks at the G_1/S boundary.

In addition to the results obtained in previous studies, the present findings indicate a clear correlation between the degree of cytotoxicity and the pattern of cell-cycle perturbations in head and neck carcinomas treated with cisplatin. If the same dependence could be confirmed in human patients, flow-cytometric analyses of tumor biopsies could become a useful means of monitoring clinical chemotherapy with cisplatin and, moreover, could improve the scheduled application of other antitumor agents, especially of bleomycin and radiation therapy, which are known to be highly effective against cells in the late G_1 und early S phases of the cell cycle [5]. On the other hand, it would be interesting to examine the extent to which cytokinetic analyses of antitumor effects induced by platinum complexes in vitro could be used to predict tumor response under in vivo conditions. Experimental investigations evaluating this possibility are under way.

Acknowledgements. The authors are indebted to Prof. Dr. T. Diamantstein, Klinikum Steglitz, Freie Universität Berlin, for stimulating discussions. Thanks are also due Mrs. K. Dunckelmann, Mrs. B. Kolon and Mrs. B. Söhl for their assistance in the experiments.

References

1. Barlogie B, Drewinko B (1977) Cell cycle-related induction of cell progression delay. In: M. D. Anderson Hospital and Tumor Clinic (eds) Growth kinetics and biochemical regulation of normal and malignant cells. Williams and Wilkins, Baltimore, pp 315–327
2. Bergerat J-P, Barlogie B, Göhde W, Johnston DA, Drewinko B (1979) In vitro cytokinetic response of human colon cancer cells to *cis*-dichlorodiammineplatinum(II). Cancer Res 39: 4356–4363
3. Braakhuis BJM, Schoevers EJ, Heinermann G, Sneeuwloper G, Snow GB (1983) Chemotherapy of human head and neck cancer xenografts with three clinically active drugs: cis-platinum, bleomycin and methotrexate. Br J Cancer 48: 711–716

4. Eastman A, Schule N (1988) Enhanced DNA repair as a mechanism of resistance to *cis*-diamminedichloroplatinum(II). Biochemistry 27: 4730–4734
5. Göhde W, Schumann J, Büchner T, Barlogie B (1975) Influence of irradiation and cytostatic drugs on proliferation patterns of tumor cells. Pulse Cytophotometry 2: 138–152
6. Harder HC, Rosenberg B (1970) Inhibitory effects of antitumor platinum compounds on DNA, RNA and protein syntheses in mammalian cells in vitro. Int J Cancer 6: 207–216
7. Harder SC, Smith RG, Leroy RF (1976) Template primer inactivation by *cis*- and *trans*-dichlorodiammineplatinum for human DNA polymerase α, β, and Rauscher murine leukemia virus reverse transciptase, as a mechanism of cytotoxicity. Cancer Res 36: 3821–3829
8. Hartog JHJ, Altona C, Boom JH van, Marcelis ATM, Marel GA van der, Rinkel LJ, Wille-Hazeleger G, Reedijk J (1983) Cis-platinum induced distortions in DNA. Eur J Biochem 134: 485–495
9. Heyden HW von, Schröder M, Achterrath W (1980) Chemotherapie von Tumoren im Kopf-Halsbereich mit Cisplatin. In: Seeber S, Schmidt CG, Nagel G, Achterrath W (eds) Cisplatin – derzeitiger Stand und neue Entwicklungen in der Chemotherapie maligner Neoplasien. Karger, Basel, pp 127–130
10. Heyden HW von, Schröder M, Lindemaier G, Beyer H-J, Klee M, Kehl A, Nagel GA (1980) Stand der zytostatischen Therapie der Plattenepithelkarzinome im Bereich des Kopfes und Halses. In: Seeber S, Schmidt CG, Nagel G, Achterrath W (eds) Cisplatin – derzeitiger Stand und neue Entwicklungen in der Chemotherapie maligner Neoplasien. Karger, Basel, pp 116–126
11. Howle JA, Gale GR (1970) *cis*-Dichlorodiammineplatinum(II): persistent and selective inhibition of deoxyribonucleic acid synthesis in vivo. Biochem Pharmacol 19: 2757–2762
12. Jacobs C, Bertino JR, Goffinet DR, Fee WE, Goode RI (1978) 24-hour infusion of cis-platinum in head and neck cancers. Cancer 42: 2135–2140
13. Kerrison JS, Sadler PJ (1977) Solvolysis of *cis*-$[Pt(NH_3)_2Cl_2]$ in dimethyl sulphoxide and reactions of glycine with $[PtCl_3(Me_2SO)]$ as probed by ^{195}Pt nuclear magnetic resonance shifts and ^{195}Pt-^{15}N coupling constants. J Chem Soc [D]: 861–863
14. Köpf-Maier P, Köpf H (1986) Cytostatische Platin-Komplexe: eine unerwartete Entdeckung mit weitreichenden Konsequenzen. Naturwissenschaften 73: 239–247
15. Köpf-Maier P, Wagner W, Liss E (1983) Induction of cell arrest at G_1/S and in G_2 after treatment of Ehrlich ascites tumor cells with metallocene dichlorides and cis-platinum in vitro. J Cancer Res Clin Oncol 106: 44–52
16. Loehrer PJ, Einhorn LH (1984) Cisplatin. Ann Intern Med 100: 704–713
17. Pascoe JP, Roberts JJ (1974) Interactions between mammalian cell DNA and inorganic platinum compounds: I. DNA interstrand cross-linking and cytotoxic properties of platinum(II) compounds. Biochem Pharmacol 23: 1345–1357
18. Pinto AL, Lippard SJ (1985) Binding of the antitumor drug *cis*-diamminedichloroplatinum(II) (cisplatin) to DNA. Biochim Biophys Acta 780: 167–180
19. Pinto AL, Lippard SJ (1985) Sequence-dependent termination of in vitro DNA synthesis by *cis*- and *trans*-diamminedichloroplatinum(II). Proc Natl Acad Sci USA 82: 4616–4619
20. Randolph VL, Vallejo A, Spiro RH, Shah J, Strong EW, Huvos AG, Wittes RE (1978) Combination therapy of advanced head and neck cancer. Cancer 41: 460–467
21. Rao PN (1980) The molecular basis of drug-induced G_2 arrest in mammalian cells. Mol Cell Biochem 29: 47–57
22. Reedijk J, Lohmann PHM (1985) Cisplatin: synthesis, antitumor activity and mechanism of action. Pharm Weekbl [Sci] 7: 173–180
23. Rosenberg B, Van Camp L, Krigas T (1965) Inhibition of cell division in *Escherichia coli* by electrolysis products from a platinum electrode. Nature 205: 698–699
24. Sorenson CM, Eastman A (1988) Influence of *cis*-diamminedichloroplatinum(II) on DNA synthesis and cell cycle progression in excision repair proficient and deficient Chinese hamster ovary cells. Cancer Res 48: 6703–6707
25. Sorenson CM, Eastman A (1988) Mechanism of *cis*-diamminedichloroplatinum(II)-induced cytotoxicity; role of G_2 arrest and DNA double-strand breaks. Cancer Res 48: 4484–4488
26. Taylor SG (1979) Head and neck cancer. In: Pinedo HM (ed) Cancer chemotherapy. Excerpta Medica, Amsterdam, pp 237–256
27. Warenius HM (1980) Identification and separation of mouse and human components of heterotransplanted human tumors. In: Sparrow A (ed) Immunodeficient animals for cancer research. MacMillan, London, pp 207–220
28. Wennerberg J, Alm P, Biörklund A, Killander D, Langström E, Tropè C (1984) Cell cycle perturbations in heterotransplanted squamous-cell carcinoma of the head and neck after mitomycin C and cisplatin treatment. Int J Cancer 33: 213–222
29. Zwelling LA, Kohn KW (1979) Mechanism of action of *cis*-dichlorodiammineplatinum(II). Cancer Treat Rep 63: 1439–1444