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EFFECTS OF TEMPERATURE ON THE INTERACTION OF CISPLATIN AND CARBOPLATIN WITH CELLULAR DNA

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Abstract—Increased levels of cisplatin (cDDP)- and carboplatin (CBDCA)-DNA adducts were detected in cDDP (10 μM)- and CBDCA (6 mM)-treated CC531 cells when the temperature was raised from 37° to 43°. In the case of cDDP, increased DNA adduct formation was already detectable at 38.5°; additional temperature steps led to further increases in DNA modification. Increased CBDCA–DNA adduct formation was observed only at temperatures higher than 40°. *In vitro* studies on the interaction of CDDP and CBDCA with isolated salmon sperm DNA, however, demonstrated no significant differences in the DNA binding rate between 37° and 43° for cDDP and a minor effect for CBDCA only at 43°, almost totally excluding a direct temperature effect on DNA platination in this temperature range. Furthermore, neither the stability of the formed platinum–DNA adducts nor the rate of adduct loss in CC531 cells was changed at higher temperatures. The observed difference in cellular adduct formation, however, could be related to increased uptake of cDDP and CBDCA into CC531 cells at higher temperatures. In the case of cDDP, a temperature shift from 37° to 38.5° resulted in a significantly higher intracellular platinum concentration (0.03 ± 0.01 vs 0.071 ± 0.021 μg platinum/10^6 cells, respectively); for CBDCA, temperatures ≥41.5° were needed to increase the platinum concentration significantly above 37° values (0.3 ± 0.1 vs 0.6 ± 0.1 μg platinum/10^6 cells, respectively). In addition, the increase in DNA adduct formation of cDDP and CBDCA at elevated temperatures was comparable with the increase in cDDP–DNA adducts after a cDDP concentration escalation at 37°, indicating a concentration-dependent increase in cDDP–DNA adducts. It seems that heat affects primarily the cellular uptake of cDDP and CBDCA and not their covalent binding to DNA.

The antitumor action of cisplatin (cDDP)§ and carboplatin (CBDCA) is thought to be mediated by binding of the drugs to DNA. Both drugs bind preferentially to GC rich DNA regions [1–4]. Important differences between cDDP and CBDCA concern the rate of interaction with DNA and the nature of the expected monofunctional adduct [5–7]. The rate constant of drug binding in solution can be up to a hundred times lower for CBDCA than for cDDP [5, 8]. The first step in the reaction of CBDCA with DNA is the formation of a monofunctional adduct [9]; the final step results in bifunctional DNA binding [5].

Intensive studies on the interaction between platinum compounds and DNA have indicated relationships between adduct formation and cell kill [10–12]. Recently, a study by Terheggen and coworkers demonstrated a correlation between the incubation concentration of cDDP, cDDP–DNA adduct formation and survival [13], while in other studies cell kill was related with the uptake of cDDP by cultured cell lines [12, 14, 15], the binding of cDDP to DNA in cultured tumor cells [15, 16], and the formation of *cis*-$Pt(NH_3)_2d(pGpG)$ adducts in human leukocytes *in vivo* [17]. Reports on cDDP-sensitive and -resistant cell lines also indicated that cell kill increases with increasing levels of DNA adducts [18, 19]. Less pronounced relationships between sensitivity and DNA modifications have also been reported [5, 16, 20]. In these cases, other factors such as DNA repair capacity and/or the ability of DNA polymerase to pass platinum-DNA might be important.

In view of the central role of the platinum–DNA interaction in cDDP- and CBDCA-induced cytotoxicity, increased levels of DNA binding by these drugs *in vivo* may result in better tumor responses. It appears that this can be achieved through heating. Heat is known to potentiate the cytotoxic effects of cDDP and CBDCA in cultured cells and in experimental animals [21–26]. Possible explanations include increased cell membrane permeability, differential changes in active membrane transport of drugs, and altered cellular metabolism [27, 28]. This might result in increased drug–DNA interaction and inhibition of DNA repair [29]. In this study, we determined the effect of heat on the binding of cDDP and CBDCA to DNA in relation to intracellular platinum concentration and survival. Quantitation of platinum–DNA adduct

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§ Abbreviations: cDDP, cisplatin, *cis*-diamminedichloroplatinum(II); CBDCA, carboplatin, *cis*-diammine(1,1-cyclobutanedicarboxylate)platinum(II); PAP, peroxidase-anti-peroxidase; PBS, phosphate-buffered saline; FAAS, flameless atomic absorption spectroscopy; and DMEM, Dulbecco's Modified Eagle's Medium.

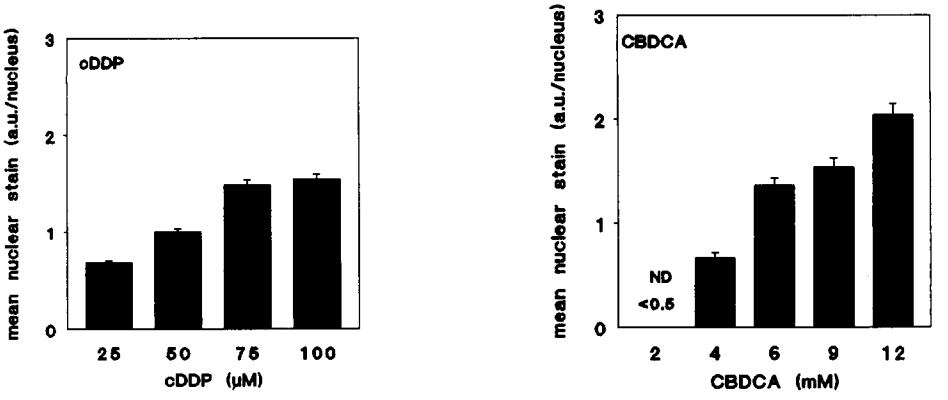

Fig. 1. Adduct-specific nuclear stain of CC531 cells directly after incubation at 37° with cDDP or CBDCA for 1 and 2 hr, respectively. CC531 cells were stained for drug-induced DNA modifications. Each bar represents the mean ± SEM of at least three independently stained slides with 30–40 nuclei each. The increase in drug-induced DNA modification was statistically significant for every increase in concentration ($P < 0.01$).

interaction products was achieved by an immunocytochemical peroxidase assay [30, 31].

MATERIALS AND METHODS

Cell line. The CC531 is a rat colonic adenocarcinoma [32] with a doubling time *in vitro* of 16 hr. Cells were cultured at 37° under 5% CO_2 in 175 cm^2 flasks (Falcon, Oxnard, CA, U.S.A.) with Dulbecco's Modified Eagle's Medium (DMEM; Irvine, U.K.) containing 10% fetal bovine serum (FBS; Gibco). Cells were subcultured after reaching a density of $5 \times 10^6/175\ cm^2$ by trypsinization and replating at a density of 10^5 cells/175 cm^2.

Drugs. *Cis*-diamminedichloroplatinum(II) (cisplatin) and *cis*-diammine(1,1-cyclobutanedicarboxylate)platinum(II) (carboplatin) were obtained from Bristol Myers (Weesp, The Netherlands) and stored in the dark at room temperature.

Drug uptake. In each experiment six flasks were used for each temperature; one flask contained about 6×10^6 CC531 cells. To each flask was added 20 mL of medium with 5 μg cDDP/mL (16 μM) or 0.74 mg CBDCA/mL (2 mM). Cells were incubated for 1 hr at 37°, 40°, 41.5° or 43°, in an incubator at 5% CO_2. After incubation, the cells were washed with phosphate-buffered saline (PBS) (two times), detached with trypsin, pooled, counted, and washed extensively with PBS. Finally, the cells were resuspended in approximately 1 mL PBS and prepared for platinum determination.

Sensitivity to cDDP and CBDCA. The sensitivity of CC531 cells to cDDP and CBDCA at different temperatures was tested by a clonogenic assay. CC531 cells were harvested as described above and counted. Cells in a single-cell suspension were plated in 6-well tissue culture clusters (Costar, Cambridge, U.K.) at 150 cells/well in conditioned medium. After 24 hr of incubation at 37°, the cells had attached to the plates and 150 μL of a cDDP or a CBDCA solution was added to get the final concentrations described under Results. The cultures were incubated at 37°, 40°, 41.5° or 43° for 75 min. The first 15 min were needed to increase the temperature from 37° up to the desired temperature. After incubation, cells were washed two times with PBS (37°), and 3 mL of fresh medium (37°) was added. All plates were returned to the 37° incubator and left for 7–10 days for the development of colonies. Colonies were fixed with ethanol, stained with crystal violet for 10 min, counted, and related to the control.

cDDP- and CBDCA-DNA adduct formation. CC531 cells were cultured on glass slides (2.6 × 6 cm) coated with ovalbumin (100 μL of 0.5% ovalbumin per slide) and incubated with cDDP (25–100 μM) or CBDCA (2–12 mM) at different temperatures (37°, 38.5°, 40°, 41.5° and 43°) for 1 or 2 hr, respectively. Cells were fixed by successive incubations in cold (−20°) 100% methanol (10 min) and acetone (2 min) and air-dried.

The presence of platinated DNA was visualized by a double peroxidase-anti-peroxidase (PAP) staining. The characteristics of the rabbit antiserum NKI-A59 against cDDP-modified calf thymus DNA (platinum/nucleotide ratio 6.7×10^{-2}) have been described by Terheggen *et al.* [33]. This antiserum also recognizes CBDCA-modified DNA. NKI-A59 (applied without further purification), goat anti-rabbit immunoglobulin (Campro Benelux, Elst, The Netherlands) and preoxidase-(rabbit)anti-peroxidase complex (American Qualex, La Miranda, CA, U.S.A.) were used in the following dilutions: 1:1800, 1:600 and 1:3000, respectively. All sera were diluted in phosphate buffer containing 10 mM KH_2PO_4, 140 mM NaCl, 10% FBS and 0.04% Triton X-100 (BDH, Poole, U.K.), as described by Terheggen *et al.* [34].

The mean nuclear stain of individual nuclei was analyzed and quantified with a Knott (München, Germany) light-measuring device with a beam diameter of 5 μm, which was coupled to a Leitz Orthoplan microscope. Data were analyzed by an Atari ST computer (Sunnyvale, CA, U.S.A.) programmed with a version of the Histochemical

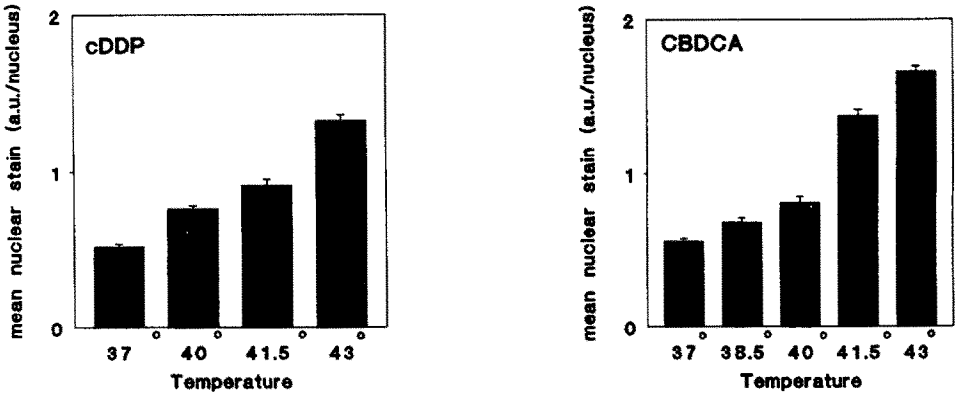

Fig. 2. Adduct specific nuclear stain of CC531 cells directly after incubation with cDDP (10 μM) or CBDCA (6 mM) at 37°, 38.5°, 40°, 41.5° and 43° for 1 hr. CC531 cells were stained for cDDP- or CBDCA–DNA adducts. Each bar represents the mean ± SEM of at least three independently stained slides with 30–40 nuclei each. The increase in DNA adduct formation after cDDP treatment was significant for temperatures $\geq$ 40° ($P < 0.001$). A significant increase after CBDCA treatment was observed between 37° and 40°, between 37° and 41.5°, and between 37° and 43° ($P < 0.01$, $P < 0.001$ and $P < 0.001$, respectively).

Data Acquisition System (Hidacsys; Microscan, Leiden, The Netherlands). The nuclear stain per scanned area was determined and expressed in arbitrary units [35]. In each slide the mean nuclear stain of 3 to 4 randomly selected areas, corresponding to 20–40 nuclei each, was measured.

The effect of heat on DNA adduct formation was studied by the incubation of CC531 cells with 10 μM cDDP or 6 mM CBDCA at different temperatures (37°, 38.5°, 40°, 41.5° and 43°) as described above. In a separate experiment, the stability of the cDDP- or CBDCA–DNA adducts was studied by the determination of adduct levels at different temperatures (37° and 43°) and up to 48 hr after drug removal. In the latter experiment, CC531 cells were incubated with CBDCA (2 mM, 2 hr) or cDDP (1 hr) at 37° or 43°; cDDP concentrations were 75 μM and 25 μM for the incubations at 37° and 43°, respectively. The relatively high concentration of 75 μM was chosen to ensure that the staining signal after repair would not be below the detection limit (staining density/nucleus < 0.5 arbitrary units [a.u.]). The dose of 25 μM at 43° was selected to stay within the linear range of the relationship between the number of cDDP–DNA adducts and nuclear stain, i.e. 0.5 and 4 a.u./nucleus).

After the drug incubation period, cDDP and CBDCA were removed by washing the cells two times with PBS, and then 3 mL of fresh medium was added. At given time points cells were fixed and prepared for immunocytochemical analysis of DNA adducts.

The existence of a correlation between cytotoxicity and adduct specific nuclear stain at different temperatures was investigated at doses of 25 μM cDDP and 1 and 6 mM CBDCA (survival and adduct formation, respectively).

Binding kinetics of cDDP and CBDCA to DNA in solution. Isolated salmon sperm DNA at a concentration of 1 mg/mL in phosphate buffer (0.1 M NaH_2PO_4, pH 7.0) was incubated with equimolar concentrations of cDDP and CBDCA (0.5 mM) in serum-free medium [6]. At fixed time points (0, 4, 24 and 72 hr) DNA samples were taken, purified from free drug by Sephadex G-50 gel filtration [5], and prepared for platinum determination.

Platinum detection by flameless atomic absorption spectroscopy (FAAS). A model AA40 Atomic Absorption Spectrometer with GTA 96 Graphite Tube Atomizer (with Zeeman background correction) from Varian (Victoria, Australia) was used to determine platinum concentrations in tumor cells and the amounts of platinum bound to salmon sperm DNA. Before samples were prepared for platinum detection, tumor cells and salmon sperm DNA were washed extensively with PBS. Sample preparation and the FAAS procedure have been described in detail elsewhere [36].

Statistics. Student's *t*-test and the Wilcoxon test were used to test for differences; P values < 0.05 were considered to indicate significance.

RESULTS

cDDP- and CBDCA–DNA adduct formation in CC531 cells. The data shown in Fig. 1 represent the cDDP- and CBDCA–DNA adduct formation in CC531 tumor cells after incubation with various drug concentrations at 37°. To achieve similar staining density, CC531 cells had to be incubated with about 100-fold higher CBDCA than cDDP concentrations. No significant nuclear stain could be determined with a CBDCA concentration lower than 2 mM. Figure 1 further demonstrates that the nuclear stain increased with increased incubation concentration level for both drugs, suggesting an approximately linear relationship between concentration and adduct formation.

When CC531 cells were treated with cDDP (10 μM) or CBDCA (6 mM) at temperatures raising

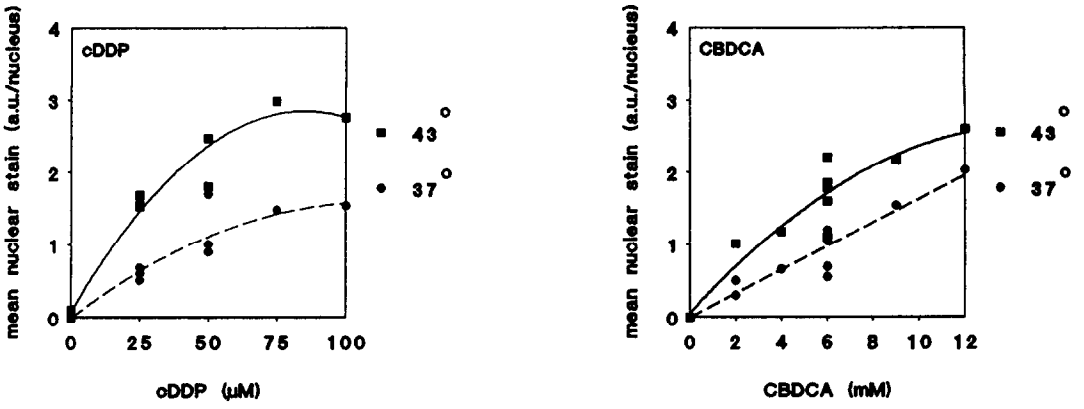

Fig. 3. Correlation between the adduct-specific nuclear stain (expressed in arbitrary units [a.u.]) of CC531 cells and the extracellular concentration of cDDP or CBDCA during incubation at 37° and 43°. CC531 cells were stained for cDDP- or CBDCA–DNA adduct formation. Each point represents the means of at least three independently stained slides with 30–40 nuclei each. For both drugs, points at 37° differed significantly ($P < 0.05$ Wilcoxon test) from the ones at 43°.

from 37° to 43°, the staining density increased significantly with increasing temperature for both cDDP and CBDCA, indicating increased levels of cDDP- and CBDCA–DNA adducts (Fig. 2). The increase in cDDP–DNA adduct level did not vary much for every temperature step. The increase in CBDCA–DNA adduct formation, however, seemed to be more pronounced at temperatures beyond 40°. The latter might indicate a difference in the binding kinetics of cDDP and CBDCA to DNA.

Comparing the concentration response at 37° and 43°, an increase of the staining density with increasing drug concentrations for both cDDP and CBDCA was demonstrated. For each concentration, the staining density was significantly higher at 43° than at 37° (Fig. 3). The percentage increase with temperature was higher for cDDP-treated cells than for CBDCA-treated cells.

To test the effect of temperature on adduct formation and adduct stability, intracellular adduct levels were followed for a period of 24 hr for cDDP and 72 hr for CBDCA. CC531 cells were incubated with cDDP at 37° (75 μM) and 43° (25 μM), or with 2 mM CBDCA at 37° and 43°. Figure 4 shows adduct kinetics after a 1-hr cDDP treatment. The nuclear stain increased between 0 and 4–6 hr after treatment at 37°, followed by a decrease between 6 and 24 hr. After incubation with cDDP at 43°, the nuclear stain increased during the first 2 hr and then decreased at approximately the same rate as at 37°. Since the incubation concentration was three times lower at 43° than at 37° (25 vs 75 μM), the efficiency of cDDP–DNA adduct formation was increased markedly at the higher temperature. The maximal adduct level at 43° occurred appreciably faster than at 37° (peak values at 2 and 6 hr, respectively). The stability of cDDP–DNA adducts, however, was not affected.

Figure 4 also illustrates adduct kinetics after CBDCA incubation. The patterns of CBDCA–DNA adduct levels in time at 37° and 43° were similar. The staining density increased up to 48 hr post-treatment and then decreased slightly at about the same rate. Nuclear stain was higher at any time after treatment at 43°, underlining the hypothesis that heat substantially increases the formation of CBDCA–DNA adducts.

Binding kinetics of cDDP and CBDCA to DNA. The binding kinetics of cDDP and CBDCA to DNA were studied in more detail in a DNA-containing solution. cDDP and CBDCA were reacted with isolated salmon sperm DNA for 0–72 hr at different temperatures, free platinum was removed, and the platinum–DNA binding was measured by FAAS. Figure 5 showed that much less CBDCA than cDDP bound to DNA after 4, 24 and 72 hr of treatment. Furthermore, a time-dependent increase of cDDP binding to DNA was observed, but no effect of the temperatures on the extent of DNA platination was detected. In the case of CBDCA, a time-dependent increase in DNA binding was also observed. The extent of CBDCA binding to DNA was not increased markedly at either 40° or 41.5° but only at 43°. This suggests that the interaction between CBDCA and DNA was slightly more temperature dependent than that between cDDP and DNA. Similar effects as described for the binding to DNA were found for the binding of cDDP and CBDCA to proteins (data not shown).

Effect of temperature on the uptake of cDDP and CBDCA. The uptake of cDDP and of CBDCA by CC531 cells increased with temperature but differed in one aspect (Fig. 6). Whereas the net uptake of cDDP was already affected by a small temperature increase over 37°, temperatures higher than 41.5° were needed to markedly increase the net uptake of CBDCA. These data indicate that heat affects uptake mechanisms of both cDDP and CBDCA but in a quantitatively different way.

cDDP- and CBDCA–DNA adduct formation and cytotoxicity. Cytotoxicity was measured by a clonogenic assay. The cytotoxic effects of both cDDP

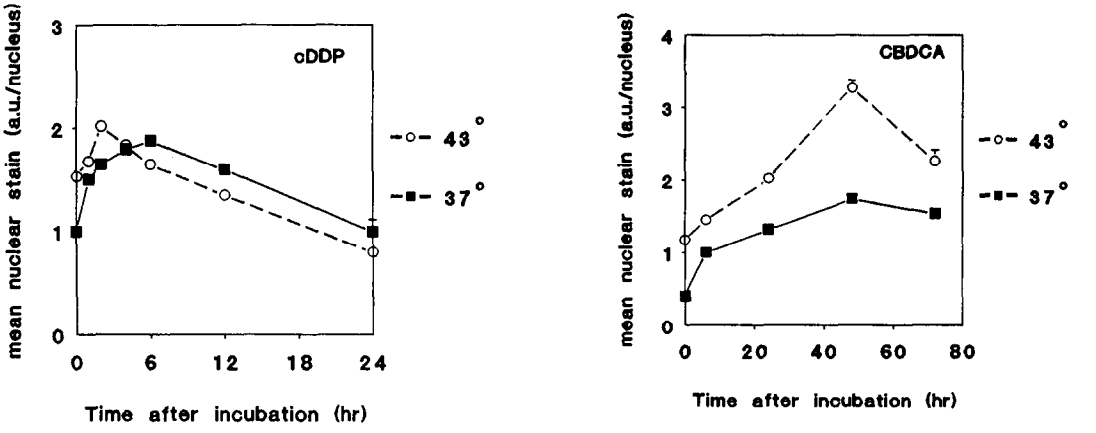

Fig. 4. Mean nuclear stain versus time after incubation of CC531 cells with cDDP at 37° (75 μM) and 43° (25 μM) or with 2 mM CBDCA at 37° and 43°. CC531 cells were stained for cDDP- and CBDCA-induced DNA modifications. Each point represents the mean ± SEM of at least three independently stained slides with 30–40 nuclei each.

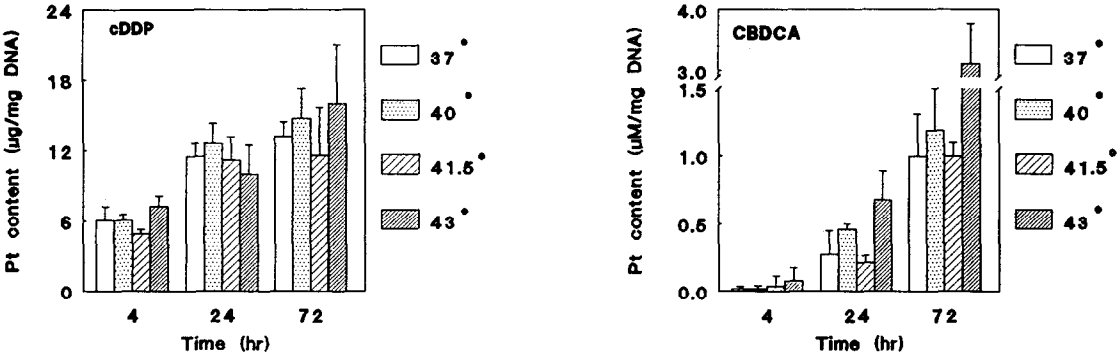

Fig. 5. Binding of cDDP and CBDCA to DNA in solution after 4, 24 and 72 hr. cDDP (0.5 mM) and CBDCA (0.5 mM) were incubated with salmon sperm DNA (1 mg/mL) at temperatures ranging from 37° to 43°. At the indicated time points, DNA samples were purified from free drug and analyzed for platinum content by FAAS. Experiments were performed in triplicate and repeated four times; each bar represents the mean ± SEM.

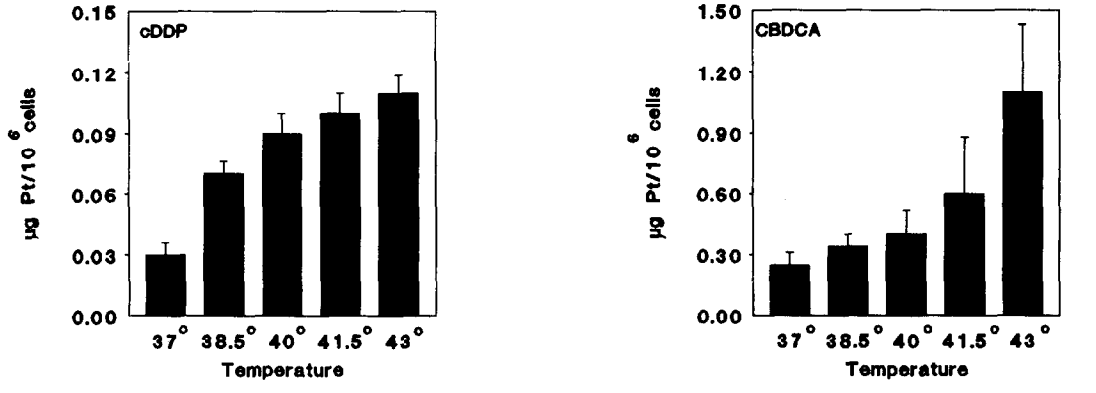

Fig. 6. Platinum concentrations in CC531 cells directly after incubation with cDDP (16 μM) or CBDCA (2 mM) at 37°, 38.5°, 40°, 41.5° and 43° for 1 hr. Each bar represents the mean ± SD of at least three experiments.

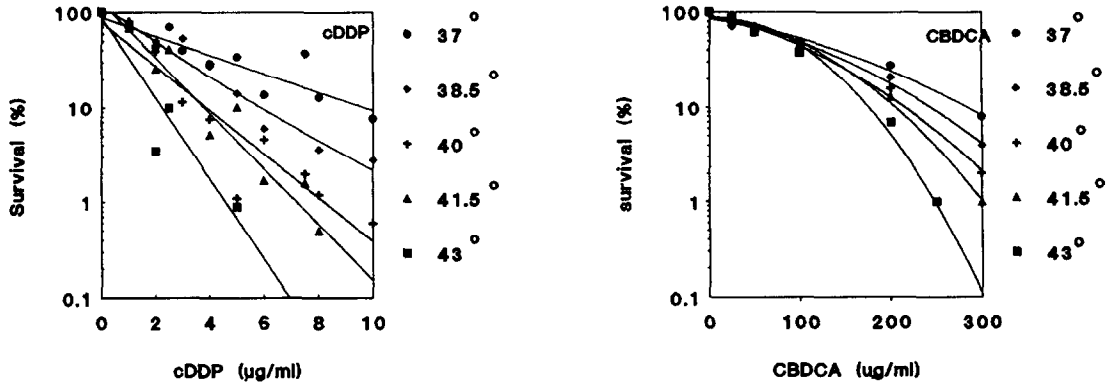

Fig. 7. Clonogenic assay of CC531 cells incubated with cDDP or CBDCA for 1 hr at 37°, 38.5°, 40°, 41.5° and 43°. All curves were adjusted to the control values at 37°, 38.5°, 40°, 41.5° and 43° and were the mean of at least five determinations. No colonies could be detected at a cDDP dose of 8 μg/mL or a CBDCA dose of 300 μg/mL at 43°.

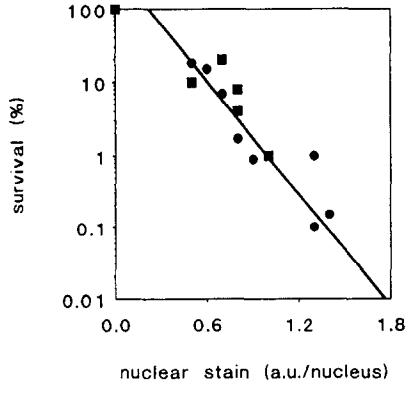

Fig. 8. Correlation between survival and the mean nuclear stain of CC531 cells after treatment with 25 μM cDDP (●) or with 1 or 6 mM CBDCA (■) at 37°, 38.5° 40° 41.5° and 43°. The best fitting curve was calculated by linear regression analysis (correlation coefficient was 0.95).

and CBDCA increased when the temperature was raised (Fig. 7). Comparing the heat effect on cytotoxicity, the increase in cytotoxicity between 37° and 41.5° was higher for cDDP than for CBDCA.

To test for a correlation between cytotoxicity and adduct formation at different temperatures, mean nuclear stain was plotted against survival (Fig. 8). Mean nuclear stain and survival were determined after incubation with cDDP (25 μM) or CBDCA (6 and 1 mM, respectively) at temperatures ranging from 37° up to 43°. Since CBDCA exposure >2 mM showed no survival of CC531 cells, survival was determined after a concentration of 1 mM. The nuclear stain of cDDP- and CBDCA-treated cells showed a good correlation with survival (correlation coefficient 0.95), indicating that an enhanced platinum–DNA adduct level results in increased cytotoxicity. The best fitting straight line ($y = -2.5x + 2.5$), calculated by linear regression analysis (Fig. 8), was comparable with that obtained by plotting the nuclear stain against a concentration-related cytotoxic response of cDDP at 37° ($y = -2.6x + 2.3$, data obtained from Figs. 1 and 7). These results indicate that heat and concentration have similar effects on DNA adduct formation and that an enhanced DNA adduct formation correlated with increased cytotoxicity.

DISCUSSION

Our recent investigations on i.p. treatment with cDDP or CBDCA combined with abdominal hyperthermia of rats with peritoneal disease [24, 25] suggested potentiation of the cytotoxicity of both drugs against CC531 peritoneal tumors. In the present study, we discuss the mechanism behind this phenomenon and stress the role of DNA platination in the potentiation of the cytotoxicity. When CC531 cells were treated *in vitro* with cDDP or CBDCA at higher temperatures, DNA platination was found to be enhanced. We investigated whether the enhanced platination of DNA was due to an increase in intracellular drug concentration or to a stimulation of drug binding to DNA. Our present data now are in favor of the former explanation.

The binding of cDDP to salmon sperm DNA in solution was not affected significantly by an increase in temperature from 37° up to 43°. Also, in the case of CBDCA the effect of temperature on the extent of the DNA binding was small, since only incubation at 43° led to a markedly higher extent of DNA binding. This means that the change in the rate of binding of cDDP between 37° and 43° was negligible, whereas that of CBDCA was just above the detection limit. In other words, temperature changes in the range of 37° and 43° did not affect the kinetics of the reaction of cDDP and CBDCA in such a way that either more cDDP/CBDCA was bound to DNA or that the binding rate was increased. In addition, the binding of CBDCA to DNA in solution was shown to be slower than that of cDDP (Fig. 5), which is in agreement with earlier reports [6, 7]. The

difference is due to the rate of binding of CBDCA, which can be up to 100 times lower than that of cDDP [5, 8]. The latter is caused by the differences in the nature of the leaving groups; the DNA-binding ligand, however, is chemically identical for cDDP and CBDCA [5]. It is to be expected, therefore, that the antiserum NKI-A59, raised against cDDP-modified DNA, also recognizes CBDCA-induced DNA modifications.

The increase in cytotoxicity of cDDP and CBDCA at higher temperatures was paralleled by an increase in the cDDP- or CBDCA–DNA adduct formation in CC531 cells. Since temperatures up to 43° slightly affected the binding of cDDP or CBDCA to DNA in solution and higher intracellular platinum concentrations were obtained after cDDP or CBDCA treatment at higher temperatures (Fig. 6), it is to be expected that the increase in cellular DNA adducts is due to the higher intracellular platinum concentrations. In the case of cDDP, higher levels of cellular DNA adducts at higher temperatures can be ascribed mainly to higher intracellular cDDP levels, while in the case of CBDCA a small part of the increased adduct formation may be due to an increase in binding *per se*. A strong argument in favor of the hypothesis that DNA platination strongly depends on the intracellular platinum concentration is the correlation between DNA adduct formation and cytotoxicity. In the case of concentration escalation and increased temperatures, the best fitting line relating DNA adduct formation with cytotoxicity almost coincided ($y = -2.6x + 2.3$ and $y = -2.5x + 2.5$, respectively). This suggests a similar mechanism for concentration escalation and increased temperatures: both lead to increased drug uptake, increased platinum–DNA adduct formation, and consequently increased cytotoxicity. There is, however, a difference between the drugs which can be traced back to the process of entering the cell. Already after the small increase in temperature from 37° to 38.5° higher intracellular platinum concentrations could be detected after cDDP treatment, leading to higher cDDP–DNA adduct formation and increased cytotoxicity. In the case of CBDCA, a small increase in temperature seemed to improve net drug uptake only marginally. An explanation for these diverging temperature dependencies might be that CBDCA is less lipophilic than cDDP, resulting in a lower capacity of CBDCA to diffuse through cell membranes [6, 36]. Temperatures above 40° seemed to increase net CBDCA uptake into CC531 cells resulting in significantly larger increments in CBDCA–DNA adduct level and cytotoxicity in the upper part of the temperature range studied.

The observed correlation between platinum–DNA adducts and cytotoxicity points to a central role of drug-induced DNA modification in cytotoxicity. Factors such as possible temperature-dependent changes in either the spectrum of DNA damage or DNA repair capacity are less likely to be involved in the more pronounced cytotoxicity at higher temperatures. This is supported by other observations. First, DNA adduct formation and loss of 43° followed a pattern comparable to that at 37° (Figs. 3 and 4). Second, the role of adduct loss (expressed as the decrease in drug-induced, adduct-specific nuclear stain) was certainly not lower at higher temperatures (Fig. 4). A comparison of cDDP–DNA adduct formation in six different cell lines and cytotoxicity (both at 37°) also demonstrates a good correlation between nuclear stain and survival [13], strengthening the correlation between platinum–DNA adduct formation and survival. Additional support for the absence of a temperature effect on the DNA platination pattern comes from the study of Fichtinger-Schepman *et al.* [37] on the effects of temperature on the reaction of DNA with cDDP *in vitro*. A variety of adducts were determined (together comprising about 90% of total DNA-bound platinum) and no alteration in the spectrum of lesions was detected between 37° and 50° [37]. The above results contrast, however, with a study on *Escherichia coli* by Brouwer *et al.* [38]. This study indicates an increase in the formation of cDDP–DNA adducts on GCG sequences but not on GAG sequences when the treatment temperature is raised from 33° to 41° [38].

Post-treatment incubation, i.e. after removal of the drug and repeated washing, showed a continuing increase in drug-induced nuclear stain for both cDDP and CBDCA. A maximal level of cDDP-induced DNA modification in CC531 cells kept at 37° was observed 6 hr after treatment. Similar increases in cDDP-induced DNA modifications have been reported by Terheggen *et al.* [13] who used the same immunocytochemical technique; these authors observed maximal nuclear stain in A2780, RIF-1 and A1847 cells at 6.5 hr post-treatment. Maximum levels of interstrand cross-links have been reached even later (between 6 and 24 hr post-treatment) with increases of up to a factor of 10 being reported [20]. It was shown that the maximal number of cDDP–DNA adducts appeared 2 hr after treatment at 43° which might be due to the higher availability of cDDP in the cytoplasm. Faster intracellular aquation of cDDP could also be an explanation, but the latter does not fit with our above conclusion that the temperature-dependent increase in DNA platination was primarily due to increased cellular drug uptake. On the other hand, processes such as altered cellular metabolism or improved cellular drug transport cannot be excluded [27, 28]. In the case of CBDCA, the rate of DNA platination at 37° mimicked the one at 43°. The only difference between the binding at these temperatures was the amount of CBDCA which bound to the DNA. The latter might be explained by the increased uptake of CBDCA into CC531 cells at 43°. A second mechanism contributing to this phenomenon might be the slightly increased capacity of CBDCA to bind to DNA at this temperature (43°).

In summary, heat in the range of 37° to 41.5° primarily influenced the uptake of cDDP and CBDCA. As a consequence of the increased drug uptake, the number of platinum–DNA adducts rose and resulted in increased cytotoxicity. The diverging effects of heat on the uptake of and DNA modification by cDDP and CBDCA may be a consequence of the difference in lipophilicity between the two drugs. It seems that the more water-soluble CBDCA needs higher temperatures to assist

membrane passage than cDDP. Results suggest that combination treatment of CBDCA with hyperthermia may need higher temperatures than cDDP to obtain similar potentiation.

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