A Rationale for Carboplatin Treatment and Abdominal Hyperthermia in Cancers Restricted to the Peritoneal Cavity

Gerrit Los, Oskar A. G. Smals, Marianne J. H. van Vugt, Martin van der Vlist, Leo den Engelse, J. Gordon McVie, and H. M. Pinedo

ABSTRACT

The purpose of this study was to optimize the treatment of cancers restricted to the peritoneal cavity by combining i.p. chemotherapy with abdominal hyperthermia. In vitro experiments demonstrated that the uptake of carboplatin into CC531 tumor cells was increased at temperatures higher than 41.5°C at dose levels of 5 and 50% cell kill. Carboplatin-DNA adduct formation and cytotoxicity, however, were already increased at temperatures of about 40°C, indicating that carboplatin-DNA adduct formation and consequently cytotoxicity could be enhanced by mild hyperthermia (temperatures in the range of 39–41.5°C).

CC531 tumor bearing rats were treated i.v. and i.p. with carboplatin (6.15 mg/kg) in combination with regional hyperthermia of the abdomen (41.5°C for 1 h). The mean temperature was 41.5 ± 0.3°C (SD) in the peritoneal cavity and 40.5 ± 0.3°C in the esophagus. Enhanced platinum concentrations were found in peritoneal tumors (factor 3) and in kidney, liver, spleen, and lung (a factor 2 average), after the combined i.v. or i.p. carboplatin-hyperthermia treatment. Pharmacokinetic data of i.p. CBDCA combined with hyperthermia demonstrated an increased tumor exposure for total and ultrafiltered platinum in plasma. The areas under the concentration x time curve for total platinum at 37°C and 41.5°C were 69 and 210 μM/h, respectively; for ultrafiltered platinum these values were 47 and 173 μM/h. This may have been due to a slower elimination of platinum from the blood at the higher temperature (t1/2 for total platinum 99 and 156 min at 37 and 41.5°C, respectively). The direct exposure of the tumor via the peritoneal fluid appeared to diminish, since the area under the curve for total platinum was lower at 41.5°C than at 37°C (576 μM/h versus 1255 μM/h, respectively).

Our results indicate that the advantage of adding hyperthermia is caused by an increased drug exposure of the tumor via the circulation. This was supported by the fact that platinum concentrations in peritoneal tumors after carboplatin treatment at elevated temperatures were similar for the i.p. and i.v. routes.

INTRODUCTION

The human peritoneal cavity is a common site of tumor recurrence of ovarian and gastrointestinal malignancies. After initial surgery the amount of residual tumor in the peritoneal cavity affects the final outcome of the treatment (1, 2). For this reason, and from the recognition that tumors growing within a body cavity are less well supplied by the bloodstream, i.p. chemotherapy may improve the survival and quality of life (3–5). Pharmacokinetic modeling has suggested that intracavitary administration of chemotherapy agents by peritoneal dialysis techniques might result in a significantly greater drug concentration in the peritoneal cavity than in plasma. This concentration difference offers a potential advantage in the treatment of malignancies confined to the peritoneal cavity (6–8).

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2 The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
3 This work was supported by Grant NK1 90-19 from the Dutch Cancer Society (Koningin Wilhelmina Fonds).
4 To whom all correspondence should be addressed.

cDDP is one of the most effective drugs available for i.p. treatment of ovarian carcinoma (9). Trials of single agent cDDP or cDDP based combination therapy i.p., in patients with residual small volume ovarian cancer who had failed to respond to i.v. cDDP treatment, have demonstrated beneficial clinical results; 30% complete remissions after single agent cDDP treatment (10) and 65% clinically free of disease after cDDP based combination therapy (11). We have previously demonstrated in a rat model that drug penetration from the peritoneal cavity into a rat peritoneal tumor plays a key role in achieving better responses (12–14). Absolute concentrations of platinum in i.p. tumor nodules were always higher after i.p. treatment than after i.v. treatment with cDDP (15) and the effective advantage of i.p. chemotherapy with CBDCA was accentuated at the periphery of the tumor (14–16).

CBDCA is a cDDP analogue with a more favorable toxic profile and an appreciable activity against ovarian cancer (17–19). The occurrence of dose limiting side effects such as nephrotoxicity, neurotoxicity, and vomiting associated with i.p. cDDP treatment, has led to trials of i.p. CBDCA. In these trials, slower drug elimination from the peritoneal cavity and a lower protein binding capacity of CBDCA were demonstrated (20). In spite of these pharmacological advantages, the relative amount of CBDCA penetrating into peritoneal tumors and tumor cells was far less than that observed for cDDP (9, 21).

In view of these results, an attempt to improve the penetration of CBDCA into tumor cells seemed logical. One way to achieve this goal might be the application of hyperthermia, since heat increases cell membrane permeability to drugs (22) and membrane transport of drugs (23) and alters cellular metabolism (24). In addition, overall pharmacokinetics may change, with heat affecting drug metabolism and excretion (25), all leading to increased antitumor responses (26, 27).

The purpose of this study was to assess the effect of regional heat on the efficacy of CBDCA in a rat tumor model system, in an attempt to improve the remission rate of cancers restricted to the peritoneal cavity.

MATERIALS AND METHODS

Rats and Drugs. Male WAG/Rij rats (8–12 weeks old; 250–300 g) were obtained from the animal department of the Netherlands Cancer Institute. The animals were kept in a temperature controlled room on a 12-h light-12-h darkness schedule and fed standard rat chow and tap water ad libitum. CBDCA was obtained from Bristol Myers (Weesp, the Netherlands). CBDCA containing vials were stored at room temperature.

Tumor Model. The tumor used (CC531) is a well defined transplantable rat colonic adenocarcinoma (28), with a doubling time in vitro of 16 h. The tumor grows s.c., i.p., and in vitro (12–15). In vitro, cells were cultured under 5% CO2 in 75-cm2 flasks (Falcon, Oxnard, CA).

3 The abbreviations used are: cDDP, cisplatin; CBDCA, carboplatin; AUC, area under the concentration × time curve; PBS, phosphate-buffered saline; i.t., intratumoral.
with Dulbecco's modified Eagle's medium (Irvine, Scotland), containing 10% fetal calf serum (Gibco). Cells were subcultured after reaching a density of $5 \times 10^6/175 \text{cm}^2$ by trypsinization and replated at a density of $10^6$ cells/$175 \text{cm}^2$.

### In Vitro Drug Uptake in Tumor Cells

In each experiment six flasks were used for each temperature, all containing about $6 \times 10^6$ CC531 tumor cells. To each flask were added 20 ml of conditioned medium with 5 µg (a nontoxic dose) or 200 µg of CBDCA/ml (50% inhibitory concentration). Cells (2 x 10^5 for each temperature) were incubated for 1 h at 37°C, 40°C, 41.5°C, or 43°C, all at 5% CO₂. After incubation, the cells were washed with PBS, detached with trypsin, pooled, counted, and washed twice with PBS. Finally, the cells were suspended in approximately 1 ml PBS and prepared for platinum determination.

### Sensitivity of CC531 to CBDCA

The sensitivity of CC531 cells to CBDCA at different temperatures was tested by clonogenic assay. CC531 cells were harvested as described before and counted. Cells in a single cell suspension were plated in 6-well tissue culture clusters (Costar, Cambridge, United Kingdom) at 150 cells/well in conditioned medium. After 24 h of incubation at 37°C, the cells had attached to the plates and 150 µl of a CBDCA solution were added to obtain the final concentrations described under "Results." The culture clusters were incubated at 37°C, 40°C, 41.5°C, or 43°C for 1 h and about 15 min preheating time. After incubation, the cells were washed twice with PBS and 3 ml of fresh medium were added. All plates were returned to the incubator and incubated for 7-10 days for the development of colonies. In contrast to the 24-h incubation period, cells were kept in platinum-containing medium after heat treatment for 7-10 days at 37°C for the continuous CBDCA incubations. Colonies were fixed with ethanol, stained with crystal violet for 10 min, counted, and related to the control.

### Pharmacokinetic Studies

Pharmacokinetic studies in rats were performed by cannulation of the carotid artery. The anesthetized animals were positioned with their lower part of their body in a thermostatically controlled water bath at 41.5°C. The temperature in the peritoneal cavity of the animals steadily increased from normal body temperature (around 38°C) to 41.5°C in about 30 min. The duration of the heat treatment at 41.5°C was 60 min. During treatment, i.p. temperatures were monitored every 5 min using copper constant thermocouple probes (IT-18; 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 (Sunrayve, CA) programmed with a version of the Histochemical Data Acquisition System [Hidacsys; Microscan, Leiden, the Netherlands (31)]. The integrated absorbance of a selected area was expressed in arbitrary units. In each slide the nuclear staining density of 3 to 4 randomly selected areas, corresponding to 20–40 nuclei each, was measured.

### RESULTS

#### In Vitro Studies on CBDCA

The effect of hyperthermia on the cellular uptake of CBDCA was studied in CC531 cells. Cells were incubated with 5 or 200 µg/ml CBDCA for 1 h at 37°C, 40°C, 41.5°C, or 43°C. The uptake of CBDCA did not increase significantly when the temperature was raised from 37 to 41.5°C, but at 43°C a clear increase was observed (Table 1). In contrast, the cytotoxicity was already increased at a temperature of 40°C (Fig. 1A). The effect of heat on the CBDCA cytotoxicity after prolonged exposure to the drug was less pronounced for temperatures round 40°C (Fig. 1B). The cytotoxicity of a 1-h exposure is probably due to an increased activation of CBDCA at higher temperatures. When CBDCA was given continuously, the effect of activation became less important. Only at 43°C is cell kill increased (Fig. 1B), probably as a result of the higher

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Pt concentration (µg Pt/10⁶ cells)</th>
<th>Ratio (µg Pt/10⁶ cells)</th>
<th>Ratio (°C/37°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>37</td>
<td>0.43 ± 0.09</td>
<td>23.9 ± 1.1</td>
<td>1.0</td>
</tr>
<tr>
<td>40</td>
<td>0.47 ± 0.17</td>
<td>ND</td>
<td>1.1</td>
</tr>
<tr>
<td>41.5</td>
<td>0.55 ± 0.19</td>
<td>28.3 ± 7.0</td>
<td>1.3</td>
</tr>
<tr>
<td>43</td>
<td>1.12 ± 0.33</td>
<td>61.2 ± 21</td>
<td>2.6</td>
</tr>
</tbody>
</table>

* Mean of 3 experiments ± SD; ND, not determined.
intracellular platinum concentration (Table 1).

The CBDCA-DNA adduct formation data seem to confirm the idea of increased activation (Figs. 2 and 3) because the staining density, which is a measure for the CBDCA-DNA adduct formation, increased. The staining density increased linearly with concentration (Fig. 2) and temperature (Fig. 3). The difference in adduct specific nuclear staining intensity between 37°C and 43°C (Fig. 2) is statistically significant (P < 0.05). The increase in DNA-adduct formation between 37°C and 40°C, between 37°C and 41.5°C and between 37°C and 43°C were significant (P < 0.01, P < 0.001, and P < 0.001, respectively).

Thermometry. During the in vivo hyperthermia experiments, temperatures in the esophagus, peritoneal cavity, and rectum were monitored (Fig. 4). The temperature reaches a steady state after 30 min of heating. The mean temperatures over the 1-h treatment for the peritoneal cavity, the rectum, and the esophagus were 41.5 ± 0.1°C, 41.2 ± 0.2°C, and 40.5 ± 0.3°C, respectively.

Pharmacokinetics of CBDCA. Pharmacokinetics of total and free platinum in the peritoneal cavity differed between rats treated with CBDCA alone and rats treated with CBDCA at elevated temperatures (41.5°C) (Figs. 5 and 6; Table 2). The AUC in peritoneal fluid was smaller in rats receiving abdominal hyperthermia, indicating a faster clearance of CBDCA from the peritoneal cavity. During the first hours after hyperthermia treatment the platinum concentration in the peritoneal cavity declined faster at 41.5°C than at 37°C (Figs. 5B and 6B). This is apparent from the half-lives (t1/2) of CBDCA in the peritoneal cavity, which were smaller for rats receiving heat (Table 2). In contrast with the faster clearance of CBDCA from the peritoneal cavity is the relative increase in ultrafiltered platinum in...
However, an increase in platinum concentration in peritoneal tumors by a factor of 3.5 was observed (Fig. 7), while the increase in other tissues, except for the intestines, did not exceed a factor of 2 (Table 3). Seven days (168 h) after i.p. treatment the increase for the combined hyperthermia treatment in peritoneal tumors was still a factor of 3 (Fig. 7).

To take advantage of the increase in the AUC of platinum in plasma after a hyperthermic treatment, CBDCA was administered i.v. The results, expressed as the 41.5°C/37°C ratio, demonstrate enhanced platinum concentrations in tissues after the combination treatment with abdominal hyperthermia (Table 3). Further the increase in platinum concentration was higher at 168 h after treatment than after 24 h, but the absolute platinum concentrations in nontumorous tissues after hyperthermic treatment at 41.5°C did not substantially change between 24 and 168 h (the 168 h data are not shown). The tumor platinum concentrations differed slightly (factor, 1.6) 24 h after treatment at 41.5 and 37°C (Fig. 7). It seems, however, that the platinum clearance from the peritoneal tumor was less after treatment at 41.5°C than at 37°C. The difference in platinum concentration in the tumor at 168 h was a factor 3.4 in favor of the hyperthermic treatment.

Comparison of platinum concentrations after the hyperthermic treatments in which CBDCA was given either i.v. or i.p. shows comparable values for platinum concentrations in the different tissues. In terms of tumor platinum concentrations, it seems that the i.v. CBDCA treatment combined with hyperthermia is as good as the i.p. treatment combined with abdominal hyperthermia in terms of platinum tumor concentr-

**Table 3 Platinum concentrations in tissues 24 h after combination treatment with i.p. or i.v. CBDCA and abdominal hyperthermia (41.5°C, 1 h) and single i.p. or i.v. treatment with CBDCA (6.15 mg/kg, normal body temperature)**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tissues</th>
<th>37°C</th>
<th>41.5°C</th>
<th>Ratio 41.5°C/37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>i.p.</td>
<td>Liver</td>
<td>0.6 ± 0.4</td>
<td>0.7 ± 0.1</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>Lung</td>
<td>0.3 ± 0.2</td>
<td>0.6 ± 0.1</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>0.4 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>Intestines</td>
<td>0.2 ± 0.1</td>
<td>0.7 ± 0.3</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>2.1 ± 0.4</td>
<td>4.2 ± 0.4</td>
<td>2.0</td>
</tr>
<tr>
<td>i.v.</td>
<td>Liver</td>
<td>0.6 ± 0.4</td>
<td>0.7 ± 0.1</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>Lung</td>
<td>0.3 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>0.2 ± 0.1</td>
<td>0.6 ± 0.2</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td>Intestines</td>
<td>0.5 ± 0.3</td>
<td>0.6 ± 0.3</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>2.7 ± 0.4</td>
<td>8.4 ± 1.5</td>
<td>2.0</td>
</tr>
</tbody>
</table>

* Mean of at least 3 experiments ± SD.

In Vivo Biodistribution of CBDCA. Pharmacokinetic data indicated increased exposure of the tumor to CBDCA via the blood as a possible advantage of a combined CBDCA-hyperthermia treatment. We therefore treated rats with peritoneal tumors i.p. or i.v. with CBDCA (6.15 mg/kg) at 37°C or in combination with abdominal hyperthermia (41.5°C for 1 h). Rats were killed 24 and 168 h after treatment and tissues such as peritoneal tumors, liver, kidney, intestine, spleen, and lung were collected. For i.p. administration, increased platinum concentrations were measured 24 h after hyperthermic treatment (41.5°C) in nearly all the selected tissues (Table 3; Fig. 7).
tration. More platinum, however, remains behind in the kidney after i.v. than after i.p. treatment at 43°C, 8.36 ± 1.5 versus 4.2 ± 0.4 µg platinum/g tissue after 24 h and 6.5 ± 1.6 versus 3.9 ± 2 after 168 h for i.v. and i.p. treatment, respectively. The latter may be reflected in increased toxicity.

DISCUSSION

During the past decade efforts have been made to improve current therapy for cancers restricted to the peritoneal cavity. One way of achieving this was to change the route of administration of cDDP from systemic to i.p. in patients with residual small volume ovarian cancer who failed to respond to i.v. cDDP (6, 10). In spite of the fact that i.p. drug delivery is still regarded as investigational, this experimental approach provided a beneficial effect of i.p. compared to systemic administration (8, 11, 14). Clinical data, however, indicated that after an initial improvement of the complete remission rate, most responding patients relapsed. The latter emphasized the need to improve the i.p. treatment. Since drug penetration from the peritoneal cavity into the tumor is one of the major advantages of i.p. treatment, it seems logical to concentrate on this aspect in an attempt to improve i.p. treatment. One way to increase drug penetration might be a combination with hyperthermia since the latter leads to altered membrane permeability (22). In a previous study we demonstrated increased penetration of cDDP into a peritoneal rat tumor when the i.p. treatment was combined with abdominal hyperthermia (32). Higher i.t. platinum concentrations were achieved and the penetration distance of cDDP from the periphery increased. A disadvantage of this combination was the concomitant increase in nephrotoxicity which was, however, less than for whole body hyperthermia (33). Based on these results, experiments were performed with hyperthermic CBDCA therapy.

The in vitro experiments with low and moderately toxic CBDCA doses in combination with hyperthermia resulted in a significant increase of intracellular platinum only at 43°C. In contrast, the cytotoxicity of a 1-h incubation with CBDCA was already higher at 40°C; this is in accordance with the results of Xu et al. (34) obtained with lower CBDCA concentrations. An explanation for this phenomenon of increased cytotoxicity at unchanged uptake levels may be found in the process of hydration. CBDCA itself does not significantly bind to proteins or other nucleophiles; nonenzymatic transformation gives rise to strong binding to aquated metabolites (35, 36). In this way, CBDCA binds to nucleophilic sites on DNA, causing interstrand and intrastrand cross-links (35). The extent of binding probably determines the cytotoxic effect (37, 38). Data from the present study demonstrate that heat induces higher CBDCA-DNA adduct levels. Whether this is due to activation of the hydration process or to increased permeability of the cell membrane, followed by increased intracellular platinum concentrations, is not totally clear. The data in this study certainly do not exclude a temperature dependent activation of the hydration process. In other words, relatively more CBDCA may not be significantly bound (36, 42). A change in the chemical structure of the drug might also be the reason in this case. After heating, the blood flow will return to normal but CBDCA will leave the peritoneal cavity faster because of the still present increase in membrane permeability. The $t_{1/2}B$ for both total and ultrafiltered platinum in the peritoneal cavity decreased if combined with heat. This is in contrast with data obtained with cDDP in the same model (39). It means that the tumor exposure to CBDCA in the peritoneal cavity declines which may have severe consequences for drug uptake by the tumor via the direct peritoneal route. The slower clearance of CBDCA from the plasma, however, provides a higher exposure of the tumor by the systemic circulation and may compensate for the loss of tumor exposure in the peritoneal cavity. A higher systemic exposure, however, may also have other consequences such as an increased myelosuppression.
The experiments on the tissue distribution of i.p. CBDDA when combined with elevated temperatures show an increased platinum concentration in all tissues at 24 h after treatment. The larger increase in platinum concentration in tumors is due to both the direct penetration of platinum into these abdominal tumors from the peritoneal fluid and the higher tumor exposure by the circulation. This increase in intratumoral platinum concentration might be beneficial with respect to the antitumor response. At 168 h after treatment, the absolute platinum concentrations were much lower for both the 37°C and 41.5°C treated group, but the tissue ratios between both groups were still comparable to the 24-h values.

In order to determine the role of the increased tumor exposure by the circulation, the effect of abdominal hyperthermia combined with i.v. CBDDA administration on the biodistribution of platinum in various tissues was studied. These experiments showed a rise in tumor platinum concentration at both 24 and 168 h after heat treatment compared to control (37°C). Comparing i.p. and i.v. treatment both with abdominal hyperthermia, it appears that the platinum concentrations in tumors do not differ. This indicates that the higher tumor platinum concentrations after i.p. treatment plus hyperthermia, in comparison to i.p. treatment alone, are probably due to the increased AUC in plasma. Furthermore, the platinum concentrations in the kidney differed. It seems that 24 and 168 h after treatment the absolute platinum concentration is higher i.v. than after i.p. treatment. This may have consequences for the nephrotoxicity. Thrombocytopenia on the other hand increased only slightly when CBDDA treatment was combined with abdominal hyperthermia (data not shown). It did not lead to a necessary reduction in the dose.

In summary, heat seems to increase the uptake of CBDDA into peritoneal tumors both after i.p. and i.v. treatment. The increased CBDDA-DNA adduct formation in vitro at higher temperatures might even suggest better tumor responses; however, this is still hypothetical. The only disadvantage of i.v. treatment over i.p. treatment in combination with heat would be the higher platinum concentration in the kidney after i.v. treatment which may affect renal toxicity.

ACKNOWLEDGMENTS

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roplatinum(II) and cis-diammine-(1,1-cyclobutanedicarboxylato)platinum(II) differ only in the kinetics of their interaction with DNA. Cancer Res., 46: 1972-1979, 1986.


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