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Original Paper

Intraperitoneal Cisplatin with Regional Hyperthermia in Advanced Ovarian Cancer: Pharmacokinetics and Cisplatin–DNA Adduct Formation in Patients and Ovarian Cancer Cell Lines

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The purpose of this study was to investigate the influence of hyperthermia on cisplatin pharmacokinetics and DNA adduct formation. The latter was investigated both in tumour cell lines *in vitro* and in tumour cells and buccal cells from cancer patients. The patients had advanced ovarian carcinoma and were entered into a phase I study for cytoreductive surgery followed by hyperthermia in combination with intraperitoneal cisplatin administration. The cisplatin–DNA modifications *in vivo* and *in vitro* were studied by an immunocytochemical method with the polyclonal antiserum NKI-A59. The patient samples for pharmacokinetic determinations were analysed by flameless atomic absorption spectrometry. *In vitro*, the combination of hyperthermia and cisplatin enhanced cell killing compared with either treatment alone, such that the cisplatin-resistant ovarian cell line A2780/DDP became almost as sensitive as the parent A2780 cell line (resistance factor reduced from 30 to 2 at the IC_{50}). In addition, increased cisplatin–DNA adducts were observed in the resistant cell line after the combined treatment compared with cisplatin alone. A good correlation was found between nuclear staining density and surviving fraction for all groups, indicating that the DNA adducts generated are an important determinant of toxicity and that the mechanism by which hyperthermia enhances kill is by increasing adduct levels. In the patients, the ratio of drug concentration in the peritoneal perfusate compared with that in plasma was found to be approximately 15, indicating a favourable pharmacokinetic ratio. Cisplatin–DNA adduct formation in tumour cells from patients was higher than in buccal cells, reflecting this higher drug exposure, i.e. local plus systemic versus systemic only. In addition, the tumour cells but not buccal cells were exposed to hyperthermia. The higher number of tumour adducts also suggests that a favourable therapeutic ratio could be achieved. Platinum–DNA adduct formation was found to decrease with distance from the surface of the tumour nodules. However, at a distance of 3–5 mm, the nuclear staining density levels were still measurable and higher than in buccal cells. In conclusion, the combined pharmacokinetic and adduct data in patients support the advantages of the intraperitoneal route for drug administration, and the addition of heat. © **1998 Elsevier Science Ltd.**

Key words: hyperthermia, cisplatin–DNA adducts, pharmacokinetics, intraperitoneal, penetration depth
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INTRODUCTION

CANCER OF ovarian epithelium is the fourth most common cause of death from cancer in women and the leading cause of death from gynaecological cancer. When the disease becomes clinically manifest, in approximately 75% of women

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the tumour will have already spread from the ovaries into the abdominal cavity [1]. Current treatment is cytoreductive surgery in combination with platinum containing chemotherapy. Despite response rates of up to 80%, long-term remissions are uncommon. The five-year survival rate for patients with advanced disease is approximately 20%, and after 10 years hardly any survivors can be found [2].

The development of resistance to chemotherapy is a common problem and constitutes a major obstacle to cure. There are abundant laboratory and clinical data indicating that acquired resistance to chemotherapy in tumours is of a moderate degree and can be partially overcome by increasing the dose. The dissemination of ovarian tumours remains largely confined to the peritoneal cavity for most of their natural history [3]. This biological behaviour provides a unique opportunity to increase drug delivery selectively at the site of the tumour by direct intraperitoneal (i.p.) instillation [4]. High peritoneal drug concentrations can be reached with acceptable systemic toxicity through low systemic uptake and rapid plasma clearance [1].

Former studies have demonstrated the presumed pharmacokinetic advantage and the logistic feasibility of i.p. drug administration. However, i.p. chemotherapy remains a topic of controversy as clinical studies, mostly performed with platinum compounds, have failed to show consistently an increased antineoplastic effect compared with standard intravenous treatment whilst others have shown the opposite [5–7]. A disadvantage of i.p. treatment with cisplatin is the limited penetration depth of the drug in tissue of approximately 2.5 mm, whereby patients with tumour nodules greater than 5 mm will receive little benefit from this treatment [8, 9]. The efficacy of i.p. chemotherapy could be improved by application of hyperthermia during i.p. chemotherapy. The rationale behind this approach is the observation that heat results in an increased cell membrane permeability, differential changes in active membrane transport of drugs and altered cellular metabolism [10–13]. In studies of simultaneous treatment with hyperthermia and cisplatin of tumour cells, an increase in the number of platinum–DNA adducts and a more than additive cell kill have been shown [14–17].

In this study we investigated the influence of hyperthermia on cisplatin pharmacokinetics and DNA adduct formation in intra-abdominal tumour marker nodules and in buccal cells of ovarian cancer patients. Since enzyme-linked immunosorbent assay (ELISA) and atomic absorption methods were not capable of detecting cisplatin–DNA adducts in individual cells, we used an immunocytochemical method employing a polyclonal rabbit antiserum, NKI-A59, raised against cisplatin–DNA adducts [18–20]. We studied the penetration depth of cisplatin in tumour biopsies for comparison with earlier studies without hyperthermia. For comparison, we also investigated the effect of the combined treatment on adduct formation of an ovarian cancer cell line *in vitro* to confirm the hyperthermia enhancement in this tumour type.

MATERIALS AND METHODS

Patients and treatment

Five women with recurrent ovarian carcinoma were studied. Inclusion criteria were: histologically confirmed ovarian carcinoma confined to the peritoneal cavity; recurrence or progression of ovarian cancer after initial therapy or after previous therapy for recurrent disease; physically and mentally able to undergo aggressive cytoreductive surgery and chemo-hyperthermia; normal haematological and biochemical liver function tests as well as creatinine and creatinine clearance (Cl_{cr} ml/min). All patients gave written informed consent. The aggressive cytoreductive surgery, including resections of tumour-bearing organs and peritonectomy of tumour containing surfaces, was performed with the objective of reducing the tumour to less than 5 mm residual nodules. A tumour marker nodule was left in the abdominal cavity. The intra-operative peritoneal chemohyperthermia was started immediately after surgery. Briefly, three silicon tubes were placed in pelvic, right subphrenicus and left subphrenicus cavities. While the patient was still under general anaesthesia, the perfusate (NaCl 0.9%), which was heated to 44°C by passage through a tubular coil in a thermostatted water bath, was infused into the peritoneal cavity through an intrapelvic tube attached to a pump. Thin microthermocouples (Mallinckrodt, St. Louis, Missouri, U.S.A., accuracy 0.1°C) were placed in the inflow, outflow tubes, intra-abdominal (pelvic, diaphragm and hypogastric) and oesophagus. The inflow temperature was maintained at 42°C, resulting in an outflow temperature of approximately 40–41.5°C. The temperature in the peritoneal cavity steadily increased from 37°C to approximately 42°C in approximately 30 min. The mean abdominal temperature and plateau was 41.5°C with a range of 40–43°C. The highest patient core temperature was 39°C (oesophagus), and there was no danger of compromising renal function. When the peritoneal cavity reached a temperature of above 40°C, the drug was injected in the perfusate [31]. The first 3 patients received 50 mg/m^2, while the last 2 patients received 70 mg/m^2 cisplatin. The duration of the cisplatin perfusion at 42°C was 90 min. After that the tumour marker nodules were excised for cisplatin–DNA adduct measurement.

Collection and processing of patient samples

For cisplatin–DNA adduct formation, buccal cells, as a marker of systemic exposure, were collected by wiping the inner cheek with a cotton swab prior to cisplatin treatment (pretreatment), directly after treatment, approx 8 h after treatment and 24 h after treatment. Cells were collected in phosphate-buffered saline (PBS), centrifuged for 10 min at 1000*g*, and washed again twice with PBS. Cytospin preparations were made on saline-coated slides followed by fixation in methanol/acetone (80/20) (−20°C).

To estimate the effect of hyperthermia and cisplatin exposure on the tumour, tissue was collected before and immediately after treatment (tumour marker nodules). The samples were snap-frozen in liquid nitrogen and stored at −70°C. 10 μm sections were cut and fixed in methanol/acetone (80/20) (−20°C) on saline-coated slides. For the pharmacokinetic study, blood (anticoagulated with EDTA), peritoneal perfusate and urine samples were collected before and every 15 min during treatment. Plasma was first separated by immediate centrifugation (5 min; 1500*g*). 1 ml of the plasma was transferred directly to an MPS-1 filtration device equipped with a YMT-30-filter (Amicon Division, W.R. Grace & Co., Massachusetts, U.S.A.) and centrifuged for 10 min at 1500*g* to obtain plasma ultrafiltrate (pUF). Plasma, pUF, peritoneal perfusate and urine were stored at −20°C until analysis.

Cell lines and culture conditions

The human cisplatin-sensitive ovarian cell line A2780 was grown as a monolayer culture in DMEM with 5% fetal calf serum (FCS) (Gibco, Breda, The Netherlands), penicillin (100 U/ml) and streptomycin (100 μg/ml) in a humidified atmosphere with 5% CO_2 at 37°C. The derived cisplatin-resistant cell line A2780/DDP was grown as a monolayer culture in RPMI-1640 with 10% FCS. Cisplatin was obtained from Bristol Myers (Weesp, The Netherlands). The cells were passaged using trypsin twice a week. The cell lines were stable with respect to morphology, doubling time and DNA content during these experiments. All experiments were done within a span of 10 passages.

Drug accumulation

Approximately 1×10^6 cells were seeded into a 75 cm^2 tissue culture flasks and treated with cisplatin 2–4 days later in log phase of growth. For the dose–response experiments, cells were treated for 2 h at 37°C or 41.5°C with a graded series of cisplatin concentrations (0–250 μM). After exposure, the cells were washed with warm PBS (37°C) and further incubated with medium. Six hours after the start of the cisplatin exposure (4 h post-treatment), the cells were washed twice with cold PBS and trypsinised. For the immunocytochemical analysis, cytospin preparation were made on 2% v/v silane coated slides.

For the cell survival determinations, cells were seeded into six-well tissue culture plates and treated for 2 h at 37°C or 41.5°C with a graded series of cisplatin concentrations (0–500 μM). The cells were washed twice with warm PBS and incubated for 10–14 days for colony formation. The number of colonies was determined macroscopically and the surviving fraction (SF) expressed as the plating efficiency (colonies per cells plated) relative to that of untreated cells.

Adduct formation determination by quantitative immunocytochemistry assay

For cytospin and tumour sections, the immunocytochemistry protocol of Terheggen and associates [20] was used with some modifications to the peroxidase steps. Briefly, slides were incubated in PBS–H_2O_2 (to inactivate endogenous peroxidase), followed by 1 M KCl, proteinase K, ethanol (to denature the DNA and to increase the accessibility of the cisplatin–DNA adducts), 1% BSA (to reduce nonspecific antibody binding) and rabbit polyclonal antiserum NKI-A59 against cisplatin–DNA adducts. To visualise the adducts, a biotinylated $F(ab')_2$ fragment of swine anti-rabbit immunoglobulin (DAKO, Denmark) was used, followed by an avidin–biotin horseradish peroxidase for amplification, and 3,3,-diaminobenzidine-HCl/nickel as peroxidase substrate.

The staining intensity of individual nuclei was measured microdensitometrically with a Knott (Munich, Germany) light-measuring device (beam diameter 0.5 μm), coupled to a Leitz Orthoplan microscope. The scanning equipment was linked to an Atari ST computer (Atari, Sunnyvale), programmed with an adapted version of the Histochemical Data Acquisition System (Hidacsys, Leiden, The Netherlands). The nuclear staining density (the integrated optical density of a selected nuclear area expressed in arbitrary units) was determined on 20 nuclei per slide which were scanned [20].

Table 1. *Patients' characteristics*

Patient no.	Age	Performance status (WHO)	Histology	Stage	No. of previous chemotherapy courses
1	32	2	serous	3	3
2	33	0	serous	1	1
3	45	0	endometrioid	2	2
4	64	0	serous	2	2
5	61	0	serous	2	2

Platinum determination by flameless atomic absorption spectrometry

To quantify the cisplatin concentration in plasma, pUF, peritoneal perfusion and urine, flameless atomic absorption spectroscopy (FAAS) with a model AA-30/40 Atomic Absorption Spectrometer with a GTA 75 autosampler (Varian, Techtron Pty Ltd., Victoria, Australia) was used. Sample preparation and FAAS procedures have been described in detail elsewhere [21]. The total dose of cisplatin given to patients was based on body surface area (mg/m^2). The maximum drug concentration C_{max} (μM) in the perfusate was measured at time zero. The end concentration of cisplatin (μM at the end of the infusion) and the amount of perfusate (l) were measured at 90 min, from which the end dose of cisplatin in mg was calculated. The absorbed cisplatin dose (%) was calculated from the starting dose minus the end dose divided by the starting dose. The highest drug concentration in total and ultrafiltrable plasma was reached between 45 and 60 min. The area under the concentration–time curve (AUC) of ultrafiltrable plasma was calculated for the duration of the dwelling time (90 min) with samples taken every 15 min.

RESULTS

Patients' characteristics

The characteristics of the 5 patients are summarised in Table 1. There was a wide age range, and all patients had received at least one or more previous platinum-containing chemotherapy. The patients all had good performance status and were able to undergo aggressive cytoreductive surgery and chemo-hyperthermia. Only mild toxicity was observed.

In vitro *study*

Colony assays were performed to test the sensitivity of both cell lines to a 2-h cisplatin exposure (Figure 1(a)). 50% survival (IC_{50}) in the sensitive A2780 cell line occurred at 1.5 μM cisplatin, whereas the resistant A2780/DDP cell line was 45 μM. Treating the resistant cell line with cisplatin and hyperthermia led to increased cytotoxicity, with an IC_{50} of 3.1 μM at 41.5°C. This combined treatment was more than additive, as can be seen in Figure 1(a), in which the curve has been corrected for the heat killing alone which was approximately 15% at 41.5°C. The resistance factor was decreased from 30 to 2 at a surviving fraction of 50%.

The antiserum NKI-A59 recognises bifunctional intrastrand cisplatin–DNA adducts which comprise approximately 90% of total cisplatin–DNA adducts [22]. The nuclear staining density of nuclei with this antibody increased with cisplatin dose for both cell lines and increased more rapidly if the resistant cells were treated with hyperthermia (Figure 1b). A plot of selected nuclear staining against surviving fraction showed good correlation between these parameters (correlation coefficient $r = 0.94$, $P > 0.001$, Figure 1c). These findings suggest that for all tested cell lines, DNA adducts are an

Table 2. Pharmacokinetic characteristics

No.	Cl_{cr} (ml/min)	Dose (mg/m^2) cisplatin	Total dose (mg) $t = 0$'	Absorbed dose (mg) (% of total)	C_{max} (μM) perfusate	*C_{max} (μM) plasma total	pUF†	AUC (pUF) (h.μmol/l)‡	Platinum 24 h urine excretion (% absorbed dose)
1	103	50	90	70.1 (78%)	100	6.6	4.1	4.8	0.4§
2	80	50	85	64.75 (76.3%)	94.4	7.8	4.9	5.7	1.4§
3	85	50	100	78.3 (78.3%)	111.1	6.6	4.1	4.9	4.3
4	80	70	133	97.36 (73.4%)	147	8.6	5.6	6.4	8.4
5	80	70	130	97.36 (74.9%)	144	9.5	6.5	7.3	6.9

*C_{max} was reached after 45 min; †plasma ultrafiltate; ‡calculated during dwelling time (90 min); §collected during dwelling time (90 min).

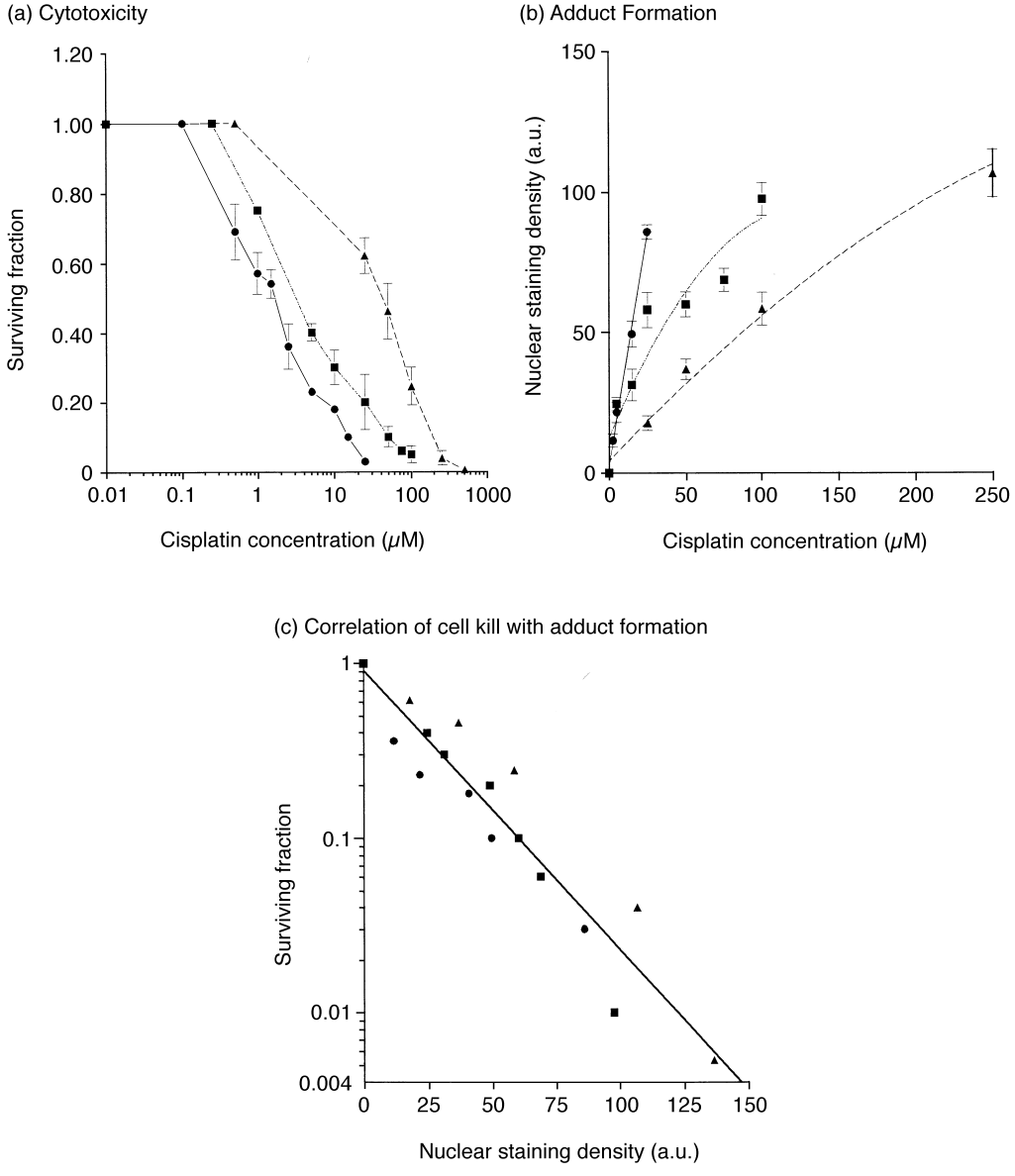

Figure 1. (a) Cytotoxicity of cisplatin in sensitive (A2780) and resistant (A2780/DDP) human ovarian carcinoma cells with cisplatin alone or combined with heat at 41.5°C (A2780/DDP). Cells were incubated with cisplatin and/or heat for 2 h. Colony numbers were determined after 8 days. All experiments were performed in duplicate. (b) Cisplatin–DNA adduct formation in drug-sensitive and -resistant cells by immunoperoxidase staining. Nuclear staining density (arbitrary units) is plotted against the concentration of cisplatin. Each point represents the mean ± SEM (bar) from 3 slides, 50 nuclei per point. (c) Correlation of cell kill with DNA adduct formation after cisplatin treatment with or without heat. Data are taken from those shown in (a) and (b). A line representing a linear regression fit through all the data is shown (correlation coefficient 0.94, $P > 0.001$). A2780 drug alone ●, A2780/DDP drug alone ▲, and A2780/DDP drug plus heat 41.5°C ■.

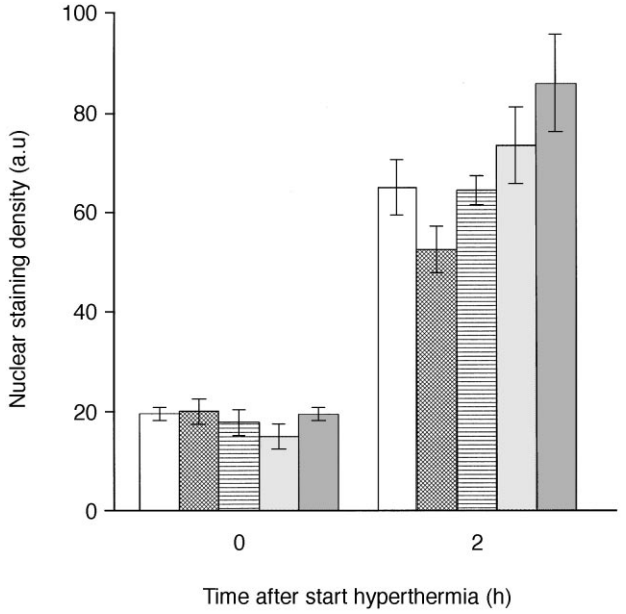

Figure 2. Cisplatin–DNA adduct formation (nuclear staining density) in tumours versus time after the start of hyperthermia. $T = 0$, the start of hyperthermia (background staining); the cisplatin infusion was begun at $t = 30'$. $T = 2$ h, the end of the combined treatment (30 min preheating and 90 min heating combined with cisplatin). Patient 1 □, Patient 2 ▨, Patient 3 ▤, Patient 4 □, Patient 5 ■. Each point represents the mean ± SEM of 2 slides; 30 nuclei per point.

important toxic lesion and that hyperthermia results in greater kill by increasing adduct levels.

Cisplatin–DNA adduct modification in tumour and buccal cells

Nuclear staining values (a.u.) of tumour cells and buccal cells before and after treatment of the patients with cisplatin are shown in Figures 2 and 3. An increase in nuclear staining density was seen in comparison with non-treated samples, for both tissues in all patients. The tumour cells showed evidence of a dose response, with higher values seen in the patients given 70 mg/m^2 (patients 4 and 5). This was less apparent in the buccal cells where there was a similar increase in all 5 patients.

Nuclear staining at different distances from the edge of the tumour showed the highest density at 0–3 mm, with

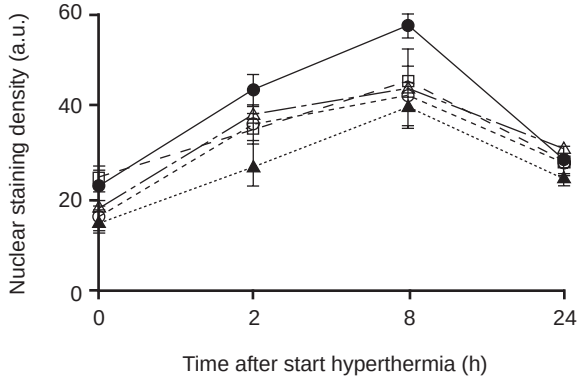

Figure 3. Cisplatin–DNA adducts in buccal cells versus time after start of treatment. Patient 1 –.△.–, Patient 2 –○–, Patient 3 –□–, Patient 4 ...▲..., Patient 5 –●–. Each point represents the mean ± SEM of 2 slides; 30 nuclei per point.

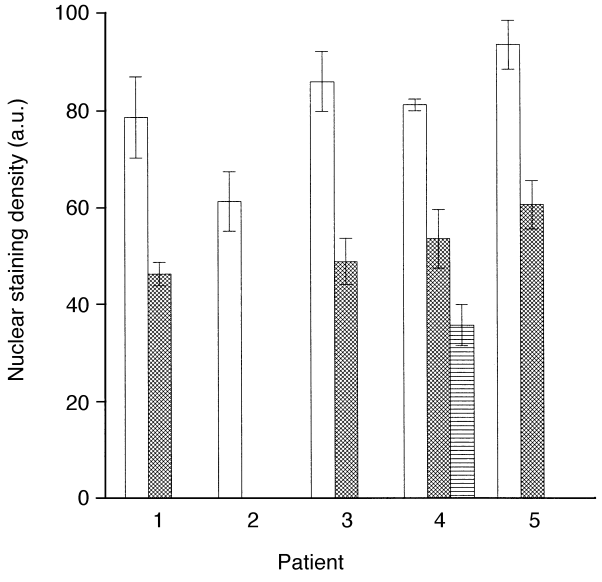

Figure 4. Adduct formation as a function of distance into the tumour nodules. Samples were collected after the end of the 2 h treatment and immunostained for platinum–DNA binding. Edge of the tumour (less than 3 mm) □, between 3 and 5 mm from the edge ▨, and > 5 mm ▤ from the edge. Each point represents the mean of 2 slides ± SEM; 30 nuclei per point.

decreased staining in the deeper regions (Figure 4). The tumour biopsy in patient 2 was smaller than 3 mm and it was therefore not possible to obtain data at 3–5 mm. The staining densities at distances of 3–5 mm and > 5 mm from the edge were lower than at the edge but still higher than the background before treatment (Figure 2). The penetration depth of cisplatin was therefore greater than 3 mm.

Pharmacokinetics

Data from 5 patients are summarised in Table 2. The absorbed dose was approximately 75% of the administered dose, which was calculated as the difference of the concentration × volume of the perfusate at the start and finish of the perfusion. The maximum concentration (C_{max}) in plasma and pUF was reached between 45 and 60 min after the beginning of the perfusion. The platinum concentration in pUF reached a peak of between 4.1 and 6.5 μM and declined during the last 15 min. The C_{max} and AUC in pUF correlated well with the absorbed dose. The ratio between the maximum drug concentration in the perfusate and plasma was 15 ± 1, implying a favourable pharmacological ratio for i.p. treatment. The amount of platinum excreted in the urine over a 24 h period was low (< 10% of the absorbed dose) at both doses.

DISCUSSION

The design of the study, which included a marker lesion, allowed cisplatin–DNA adduct formation to be examined in tumour nodules from patients. In previous rodent studies, it has been shown that high levels of drug found within the peritoneal cavity would penetrate only the superficial layer of the tumour (3 mm) leaving tumour cells in the centre undisturbed [8, 23, 24]. This low penetration was probably the reason why intraperitoneal treatment of cisplatin alone has not been effective in producing long-term survival in patients with tumour nodules greater than 5 mm diameter [4, 6].

These data in rodents were obtained using proton-induced X-ray emission (PIXE) which gives information on the total platinum concentration as a function of position in a tumour section. No data on the formation of cisplatin–DNA adducts have been reported. With the immunocytochemistry assay, it was possible to obtain information on cisplatin–DNA adduct formation at different depths, which may be more relevant for cytotoxicity than total platinum concentration. In the present study cisplatin–DNA adducts at distances greater than 3 mm from the tumour periphery were clearly present. As was shown in a rodent study as well in this study, cisplatin penetrates deeper under hyperthermic conditions [25]. At depths greater than 5 mm, nuclear staining was approximately the same as in buccal cells which received systemic exposure alone. At this depth, it was therefore not possible to discriminate whether this was due to systemic or local exposure.

Our *in vitro* study with sensitive and resistant cell lines confirmed that addition of hyperthermia to cisplatin increased the cytotoxic effect of cisplatin [15, 16, 26]. The mechanism of this enhancement is probably through increasing drug uptake by increasing cell membrane permeability and drug transport [10–12]. The cisplatin resistance of these A2780/DDP cells appears to be caused by several factors, including a 3-fold reduction in cellular drug uptake, a 2–4-fold increase in intracellular glutathione, and a 2-fold reduction in UV sensitivity, implying an enhanced capacity for nucleotide excision repair (P. van de Vaart, E. Heesbeen, The Netherlands Cancer Institute, Netherlands). These factors are not inconsistent with DNA–drug adducts being the major determinant of cell killing. The end result is a higher intracellular cisplatin level leading to an increased number of cisplatin–DNA adducts [17]. The resistant cell line became more sensitive, with the resistance factor at the IC_{50} level decreasing from 30 to 2. Combining all the *in vitro* data with and without hyperthermia for both cell lines, a high degree of correlation was shown between cell killing and the level of cisplatin–DNA adduct formation. This suggests that the adducts, measured immunocytochemically, are a major cause of toxicity and that hyperthermia allows greater numbers to be formed, hence the enhanced kill. This is consistent with a previous study with cisplatin alone by Terheggen and colleagues [20], who found such a correlation with a range of cell lines, including parent and resistant pairs. A study by Los and associates [17] with a CC531 colon cancer cell line showed equal repair for equal nuclear staining with or without hyperthermia, implying a greater role for adduct induction than adduct repair. This is also consistent with the present study.

Other investigations on platinum–DNA adduct levels in white blood cells or buccal cells of patients with different forms of cancer have shown a correlation with disease response [18, 27, 28]. A high degree of correlation was also found between the level platinum content (μg/g tumour) and nuclear staining, with a correlation coefficient of 0.62 ($P = 0.019$) [29]. Data obtained from the *in vitro* experiments cannot be directly extrapolated to patients, since there are a variety of patient-related factors, such as age, tumour type, prior treatment status, patient performance status, drug dosage, drug pharmacology, biopsy site (primary or metastatic deposit), clinical resistance and drug metabolism which could influence cisplatin–DNA binding [30, 31]. However, these *in vivo* results imply that the treatment regimen studied here fulfils at least one important condition, namely, it resulted in the binding of certain amounts of cisplatin to the cellular target i.e. DNA.

For the pharmacology of i.p. cisplatin in combination with hyperthermia, we confirmed that the pharmacological advantage of i.p. chemotherapy results from increased exposure of the peritoneal tumour without increased systemic exposure. We found a ratio between peak peritoneal drug concentration and peak plasma drug concentration of approximately 15. This was lower than that reported by other authors who found ratios between 25 and 65 [32–35]. The lower ratio is probably caused by two factors: (a) the perfusate volume in our study was 3 l of physiological saline, whereas other investigators used 1.5–2 l; (b) hyperthermia caused higher cisplatin absorption in comparison to i.p. alone [25]. The pUF levels were comparable with the literature of only cisplatin i.p. but the urine excretion of cisplatin was much lower [34]. The ratio, the absorption and the low systemic exposure suggest that a good therapeutic ratio can be obtained with this treatment. This difference in cisplatin concentration in peritoneal perfusate and serum was reflected in the nuclear staining levels in tumour nodules and buccal cells.

In summary, we have shown that cisplatin in combination with hyperthermia *in vitro* leads to a higher cisplatin–DNA adduct formation which was a good predictor of the cytotoxic effect. In patients, a level of cisplatin–DNA adduct formation, which should be sufficient to produce a cytotoxic effect, was reached in the tumour nodules to a depth of at least 5 mm. The benefit of intraperitoneal administration with combined hyperthermia treatment, with high local exposure and concomitant low systemic exposure was shown. These results provide a rationale for further exploration of this combined intraperitoneal treatment with cisplatin and hyperthermia, given the mild toxicity observed in these patients.

1. McClay EF, Howell SB. A review: intraperitoneal cisplatin in the management of patients with ovarian cancer. [review]. *Gynec Oncol* 1990, **36**(1), 1–6.
2. Neijt JP, ten Bokkel Huinink WW, van der Burg ME. Long-term survival in ovarian cancer: mature data from The Netherlands Joint Study Group for Ovarian Cancer. *Eur J Cancer* 1991, **27**, 136–172.
3. Longo DL, Young RC. The natural history and treatment of ovarian cancer. *Ann Rev Med* 1981, **32**, 475–490.
4. Markman M. Intraperitoneal cisplatin and carboplatin in the management of ovarian cancer. [review]. *Semin Oncol* 1994, **21** (2 Suppl. 2), 17–19.
5. Howell SB, Zimm S, Markman M, *et al.* Long-term survival of advanced refraction ovarian carcinoma patients with small-volume disease treated with intraperitoneal chemotherapy. *J Clin Oncol* 1987, **5**(10), 1607–1612.
6. Howell SB, Kirmani S, McClay EF, Kim S, Braly P, Plaxe S. Intraperitoneal cisplatin-based chemotherapy for ovarian carcinoma. *Semin Oncol* 1991, **18**(1 Suppl. 3), 5–10.
7. Alberts DS, Liu PY, Hannigan EV, *et al.* Phase III study of intraperitoneal (IP) cisplatin (CDDP)/intravenous (IV) cyclophosphamide (CPA) in patients with optimal disease stage III ovarian cancer: A AWOG-GOG-ECOG intergroup study. [Abstract]. *Proc ASCO* 1995, **14**, 273.
8. Los G, McVie JG. Experimental and clinical status of intraperitoneal chemotherapy. *Eur J Cancer* 1990, **26**, 755–762.
9. Markman M, Reichman B, Hakes T, *et al.* Responses to second-line cisplatin-based intraperitoneal therapy in ovarian cancer: influence of a prior response to intravenous cisplatin. *J Clin Oncol* 1991, **9**(10), 1801–1805.

10. Arancia G, Crateri trovalusci P, Mariutti G, Mondovi B. Ultrastructural changes induced by hyperthermia in chinese hamster V79 fibroblasts. *Int J Hyperthermia* 1989, **5**, 341–350.
11. Hahn GM, Shiu EC. Effect of pH and elevated temperatures on the cytotoxicity of some therapeutic agents on Chinese hamster cells *in vitro*. *Cancer Res* 1983, **43**, 5789–5791.
12. Hahn GM, Braun J, Har-Kedar I. Thermochemotherapy: synergism between hyperthermia (42–43°C) and adriamycin (or bleomycin) in mammalian cell inactivation. *PNAS* 1975, **172**, 937–940.
13. Hettinga JVE, Lemstra W, Koning AWT, Kampinga HH. Cisplatin sensitivity and thermochemosensitization in thermotolerent cDDP-sensitive and -resistant cell lines. *Br J Cancer* 1995, **71**, 498–504.
14. Ohno S, Siddik ZH, Zwelling LA, Bull JMC. Thermal enhancement of drug uptake and DNA adducts as a possible mechanism for the effect of sequenceing hyperthermia on cisplatin-induced cytotoxicity in L1210 cells. *Cancer Chemother Pharmacol* 1994, **34**, 302–306.
15. Akaboshi M, Tanaka Y, Kawai K, Akuta K, Imasunaga S, Ryu J. Effect of hyperthermia on the number of platinum atoms binding to DNA of HeLa cells treated with Pt-195m-radiolabelled *cis*-diaminedichloroplatinum(II). *Int J Radiat Biol* 1994, **66**, 215–220.
16. Los G, van Vugt MJ, Pinedo HM. Response of peritoneal solid tumours after intraperitoneal chemohyperthermia treatment with cisplatin or carboplatin. *Br J Cancer* 1994, **69**(2), 235–241.
17. Los G, van Vugt MJ, Den Engelse L, Pinedo HM. Effects of temperature on the interaction of cisplatin and carboplatin with cellular DNA. *Biochem Pharmacol* 1993, **46**(7), 1229–1237.
18. Fichtinger-Schepman AMJ, Van Oosterom AT, Lohman PHM, Berends F. cis-Diamminedichloroplatinum(II)-induced DNA adducts in peripheral leukocytes from seven cancer patients: quantitative immunochemical detection of the adduct induction and removal after a single dose of cis-diamminedichloroplatinum (II). *Cancer Res* 1987, **47**, 3000–4004.
19. Reed E, Gupta-burt S, Litterst CL, Poirier MC. Characterization of DNA damage recognized by an antiserum elicited against *cis*-diamminochloroplatinum(II)-modified DNA. *Carcinogenesis* 1990, **11**, 2117–2121.
20. Terheggen PMAB, Emondt JY, Floot BGJ, *et al.* The correlation between cell killing by *cis*-diamminedichloroplatinum(II) in six mammalian cell lines and binding of a *cis*-diamminedichloroplatinum(II)-DNA antiserum. *Cancer Res* 1990, **50**, 3556–3561.
21. Warmerdam LJC, van Tellingen O, Maes RAA, Beijnen JH. Validated method for the determination of carboplatin in biological fluids by Zeeman Atomic Absorption Spectrometry. *Fresenius J Anal Chem* 1995, **351**, 1820–1824.
22. Terheggen PMAB, Floot BGJ, Lempers ELM, van Tellingen O, Begg AC, Den Engelse L. Antibodies against cisplatin-modified DNA and cisplatin-modified (di)nucleotides. *Cancer Chemother Pharmacol* 1991, **28**(3), 185–191.
23. Los G, Mustsaers PHA, van der Vijgh WJF, Baldew GS, de Graaf PW, McVie JG. Direct diffusion of *cis*-diamminedichloroplatinum(II) in intraperitoneal tumors after intraperitoneal chemotherapy: a comparison with systemic chemotherapy. *Cancer Res* 1989, **49**, 3380–3384.
24. Los G, Mutsaers PH, Dubbelman R, van der Hoeven CJ, McVie JG. Penetration of carboplatin and cisplatin into rat peritoneal tumor nodules after intraperitoneal chemotherapy. *Cancer Chemother Pharmacol* 1991, **28**, 159–165.
25. Los G, Sminia P, Wondergem J, Mutsaers PH, *et al.* Optimisation of intraperitoneal cisplatin therapy with regional hyperthermia in rats. *Eur J Cancer* 1991, **27**(4), 472–477.
26. Nishiue T, Kojima O. Local treatment of rabbit VX2 rectal carcinoma with combined hyperthermia and intratumoral CDDP injection. *Int J Hyperthermia* 1994, **10**, 619–626.
27. Blommaert FA, Michael C, Terheggen PMAB, *et al.* Drug-induced DNA modification in buccal cells of cancer patients receiving carboplatin and cisplatin combination chemotherapy, as determined by an immunocytochemical method: inter-individual variation and correlation with disease response. *Cancer Res* 1993, **53**, 5669–5675.
28. Reed E, Parker RJ, Gill I, *et al.* Platinum–DNA adduct in leukocyte of a cohort of 49 patients with 24 different types of malignancies. *Cancer Res* 1993, **53**, 3694–3699.
29. Los G, Blommaert FA, Barton R, *et al.* Selective intra-arterial infusion of high dose platin in advanced head and neck cancer patients results in high tumor platinum concentrations and platin–DNA adduct formation. *Cancer Chemother Pharmacol* 1995, **37** (2), 150–154.
30. Selby P, Buick RN, Tannack I. A critical appraisal of the human tumor stem-cell assay. *New Engl J Med* 1983, **308**, 129–134.
31. Von Hoff DD, Clark GM, Stogdill J *et al.* Prospective clinical trial of a human tumor cloning system. *Cancer Res* 1983, **43**, 1926–1931.
32. Casper ES, Kelsen DP, Alcock NW, Lewis JL Jr. IP cisplatin in patients with malignant ascites: pharmacokinetic evaluation and comparison with the iv route. *Cancer Treat Rep* 1983, **67**(3), 235–238.
33. Pretorius RG, Hacker NF, Berek JS, *et al.* Pharmacokinetics of IP cisplatin in refractory ovarium carcinoma. *Cancer Chemother Pharmacol* 1983, **67**(12), 1085–1092.
34. Zakris L, Dewhirst MW, Hoopes PJ, Page RL, Olshen JR. Pharmacokinetics and toxicity of intraperitoneal cisplatin combined with regional hyperthermia. *J Clin Oncol* 1987, **5**(10), 1613–1620.
35. Howell SB, Pfeifle CE, Wung WE, Olshen A. Intraperitoneal *cis*-diamminechloroplatinum with systemic thiosulfate protection. *Cancer Res* 1983, **43**, 1426–1431.

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