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Platinum distribution in *intra*peritoneal tumors after *intra*peritoneal cisplatin treatment*

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Summary. The spatial distribution of platinum (Pt) in the kidney was studied by an autoradiographic technique, in which cisplatin (CDDP) was replaced by ^{195m}Pt-labeled CDDP, and by proton-induced X-ray emission (PIXE). Although both studies demonstrated comparable spatial distribution patterns, PIXE had the advantage that Pt concentrations could be determined quantitatively, in contrast to the relative information obtained by the autoradiographic technique. Using PIXE, the distribution of Pt in i.p. tumors was studied after i.p. administration of CDDP. The highest Pt concentrations were always found on the periphery of tumors, indicating that the periphery was exposed to a higher drug concentration than the center. Dose was correlated to the concentration of CDDP at both the center and the periphery ($r=0.99$). The Pt concentration in the periphery was usually higher by a factor of 2–3 after i.p. administration than after i.v. treatment, whereas in the center of the tumor no concentration difference could be detected. The penetration depth of CDDP lay between 1 and 2 mm and was calculated from the differences in Pt concentration after i.p. and i.v. treatment. This indicates that the effective advantage of i.p. chemotherapy with CDDP in cases of cancers limited to the peritoneal cavity is accentuated at the periphery of the tumor.

Introduction

Cisplatin is an established anticancer agent whose clinical success has been offset by acute and chronic toxicity [3, 12, 21]. In cases of cancers limited to the peritoneal cavity, Howell and co-workers [13] have demonstrated that delivery of CDDP by the i.p. route is feasible and well tolerated [13]; other investigators [7, 20, 28] have shown that i.p. administration ensures that the peritoneal cavity is exposed to higher drug concentrations than the rest of the body. Clinical response rates have been achieved after i.p. treatment with several cytotoxic agents [1, 31], and experimental data from the rat [19, 20] indicate that higher Pt concentrations can be detected in i.p. tumors after i.p. administration of CDDP than are achieved via the i.v. route [19, 20].

Classic pharmacokinetic studies have shown the retention in tissues [13] of at least half of every dose of the drug given to patients. Since small differences in Pt tissue concentrations might account for differential tissue toxicity [29, 30] and antitumor activity [19, 30], quantitative analysis of Pt with a low detection limit is necessary. Physical techniques available for element analysis include atomic absorption spectroscopy (AAS) and neutron activation analysis (NAA). These techniques can be used only for the analysis of bulk samples and not for studying the spatial distribution of CDDP in tissues; however, the latter can be studied with two techniques: autoradiography [15] and proton-induced X-ray emission (PIXE) [14].

After treatment of an animal with ^{195m}-Pt-containing CDDP, autoradiography on cryosections demonstrates a macro-distribution of Pt in tissues, although only relative information about the concentrations can be obtained. On the other hand, PIXE is a technique frequently applied in the biomedical field to detect trace elements on a micro scale at concentration levels of about 1 μg/g, with a spatial resolution of about 10 μm [10]. The PIXE technique has previously been used by our group [9, 14] to provide information about Pt concentrations in tissues.

The present study demonstrates the validity of the PIXE method by comparing the distribution of Pt in the kidney as measured by PIXE and autoradiography; the PIXE method was then used to demonstrate the distribution of Pt within i.p. tumors after the administration of CDDP different routes.

Materials and methods

Rats. Male WAG/Rij rats, 8–12 weeks old at the time of the experiments, were obtained from the animal department of the Netherlands Cancer Institute and kept under standard conditions.

Tumor. CC531 colonic adenocarcinoma was induced by methylazoxymethanol and is well defined [33]. The tumor grows s.c. and i.p. in vitro. CC531 adenocarcinoma is sensitive to several cytostatic drugs, including CDDP. In vitro it is replated at a density of 1×10^5 cells in fresh Dulbecco's minimum essential medium (DMEM) with 10% foetal calf serum (Flow Laboratories).

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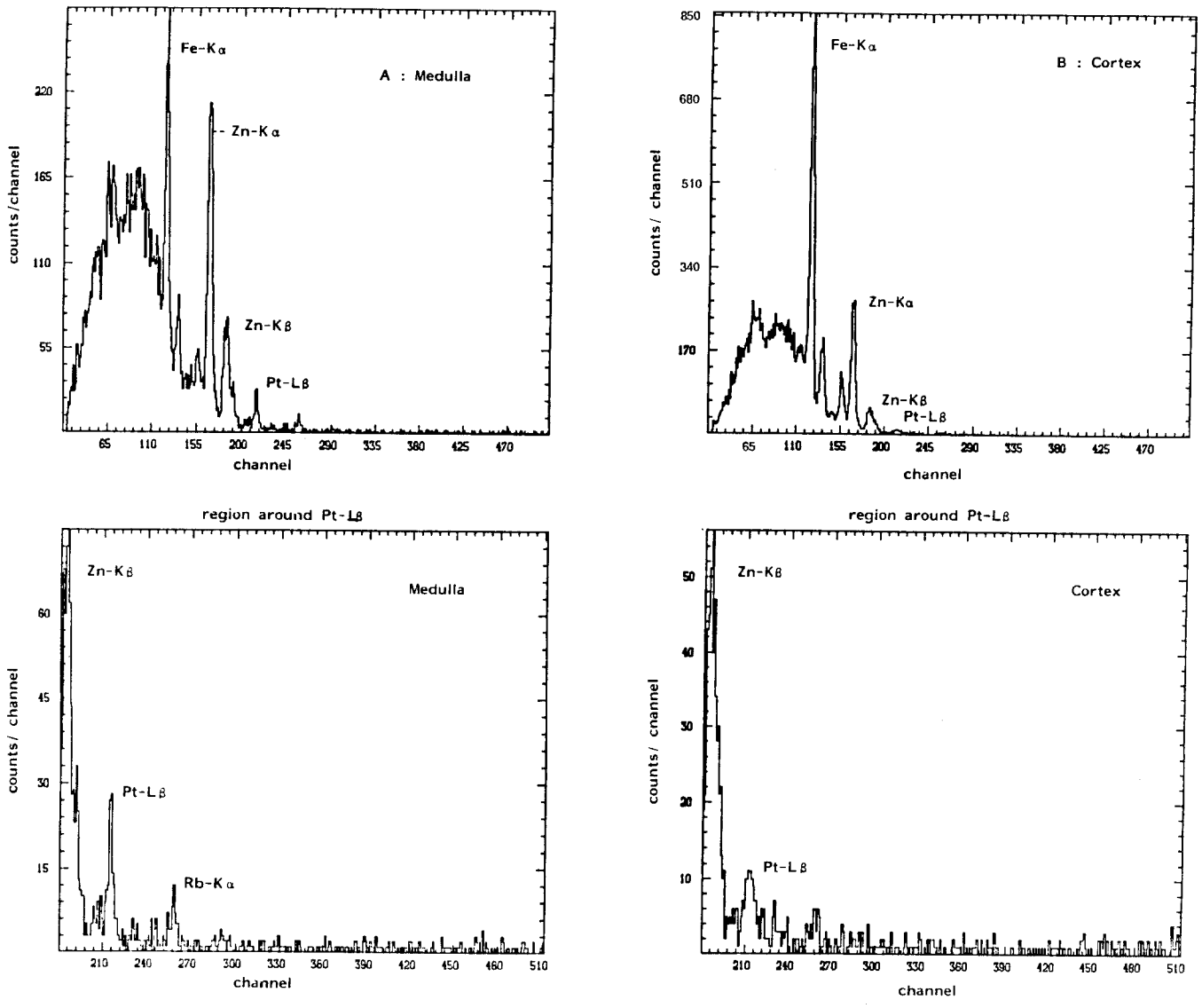

Fig. 1. Two X-ray spectra obtained with a PIXE micro-beam in a frozen kidney section. The *left panels* show. **A** the spectra of a position in the medulla; the *right panels* show **B** the spectra in the cortex. The distance between both positions is 75 μm. From both spectra a detail of the spectrum around the Pt Lβ line is demonstrated. The y axis is scaled linearly

Drugs. CDDP was made by Bristol Myers (Weesp, The Netherlands). [^{195m}Pt]-CDDP (sp. act., 30 μCi/mg) was obtained from the Interfaculty Reactor Institute (IRI) (Delft, The Netherlands).

Rat model. WAG/Rij rats were inoculated i.p. with 2×10^6 CC531 tumor cells in 2 ml phosphate-buffered solution (PBS) on day 0. After 4 weeks, small tumor nodules 2–5 mm in diameter were present in about 80% of the rats. Tumor nodules were situated on the diaphragm and peritoneum and on the mesentery between the intestines. Distant metastases were rare. Treatment with CDDP was started 28 days after inoculation. Tumors were collected at set times to determine Pt concentrations in tissues.

PIXE. The PIXE micro-beam facility at the Eindhoven University of Technology and that at the Free University in Amsterdam were used to measure Pt concentrations at different levels in tumor and kidney sections. The technical conditions have previously been described elsewhere [9, 14, 17]. For the measurement of spatial distribution in tumors, 40 μm cryostat sections were cut and, after drying (the mass thickness was about 0.5 mg/cm^2), they were covered with an aluminum foil and packed between polystyrene layers [8]. Calibration samples were prepared as follows: the polystyrene instead of a section was loaded with a well-defined solution of cobalt acetylacetonate to establish quantitatively the ratio of a well-known cobalt peak area and the maximal height of the *Bremsstrahlung* that appears predominantly at lower energies in the spectra. Pt concentrations were determined in kidneys in a line scan from the medulla into the cortex (beam diameter, 25 μm) and in tumors from the periphery into the center of the tumor (beam size, about 40-μm diameter; distance between each point measured, about 500 μm).

Autoradiography. Rats were treated i.v. with 5 mg/kg ^{195m}Pt-labelled CDDP. After 24 h the animals were

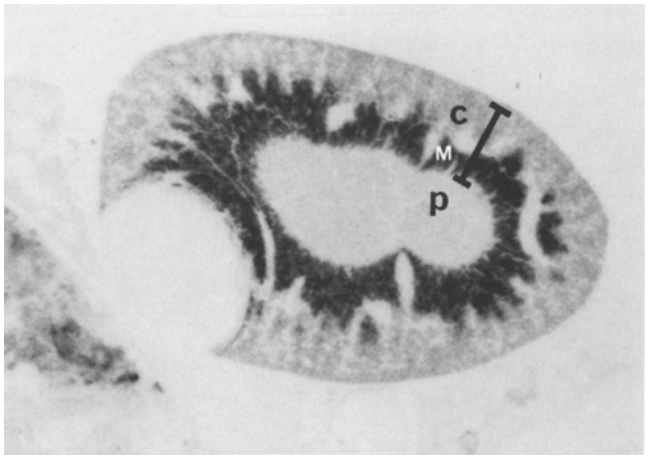

Fig. 2. A ^{195m}Pt distribution study in the kidney. A WAG/Rij rat was injected with Pt-labelld CDDP (4 mg/kg). The distribution of Pt was visualized in a frozen section. The bar indicates the line along which Pt concentrations were determined by PIXE (see Fig. 3). *P*, pelvis; *M*, medulla; *C*, cortex

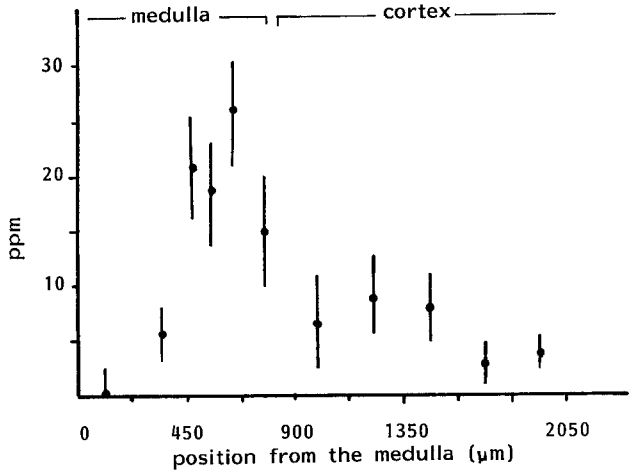

Fig. 3. Pt distribution along a line scan from the medulla into the cortex on a frozen section of the kidney. The Pt concentration (in ppm) is plotted on the y axis; on the x axis the corresponding areas in the kidney are shown expressed in µm (bars represent the SE). Two line scans, with comparable distribution patterns, were carried out, one of which is visualized in this figure

sacrificed and their kidneys, removed. Cross sections of 10-µm thickness were cut and prepared for autoradiography by standard techniques.

Pt distribution in the kidney. Rats were treated i.v. with 5 mg/kg ^{195m}-Pt-CDDP for autoradiographic studies or with 5 mg/kg CDDP for PIXE measurements. After 24 h their kidneys were removed and coronal slices were made and cryostat sections (40 µm), cut. The sections were then laid on photographic paper for measurement of radioactive Pt or on carriers for PIXE determination [14]. In the autoradiographic study Pt was visualized on an autoradiograph, whereas in the PIXE study the Pt concentration was expressed in parts per million.

Distribution of Pt in tumors. WAG/Rij rats were inoculated i.p. with CC531 tumor cells (2×10^6). After 4 weeks, rats received either one, two or three repeated doses of 4 mg/kg each, with a delay of 5 days between the doses, or two doses of 10 mg/kg each at an interval of 24 h. Tumor tissue was collected 24 h after the single injection and 48 h after the last administration of the repeated injections. The distribution of Pt was quantitatively determined by PIXE.

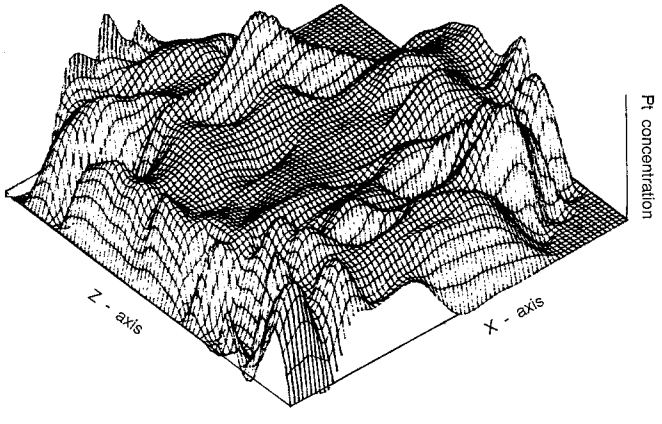

Fig. 4. A three-dimensional distribution plot of the relative Pt concentration (y axis) as a function of the position in a frozen tumor section (x, z axes). The Pt concentration was determined by PIXE

Statistics. The Wilcoxon test was used to determine significance; *P* values of > 0.05 were considered to be nonsignificant.

Results

Quantitative determination of Pt in frozen tissue sections

Micro-beam PIXE can be used to determine concentrations of different elements, including Pt, in very small volumes of tissue. In Fig. 1, two X-ray spectra at two different locations in a frozen tissue section of the kidney of a rat treated with CDDP (5 mg/kg i.v.) are presented. Pt was detected in the region around the channel numbers 200–220 (Pt-Lβ line at 11.069 keV). Figure 1 A demonstrates a Pt-Lβ peak in the medulla, with a peak height of 28 counts, and Fig. 1 B shows one of 11 counts located in the cortex. Both spectra demonstrate background noise in the range of 2–6 counts. Not shown in these spectra is the interference between the Pt-Lβ line and the Se-Kα line. Previous studies have demonstrated that the interference can be reduced by a selective germanium absorber (absorber edge, 11.103 keV) [15]. The germanium absorber suppressed the Se-Kα line at 11.210 keV and the Pt-Lβ2 line at 11.249 keV. However, the Pt-Lβ1 at line 11.069 keV was maintained upright.

A disadvantage of this absorber is the secondary fluorescence effect that yields germanium X-rays (Ge-Kβ line at 10.980 keV), which interfere with Pt-Lβ. The Ge-Kβ fluorescent peak observed in all spectra appeared to be equivalent to a concentration of about 1.5 ppm [18]. Therefore, this background was subtracted from the measured values in Fig. 1 and led to the setting of the detection limit for Pt at 2 ppm. After correction for Ge-Kβ fluorescent interference counts, the remaining spectral interferences

Table 1. Accumulation of cisplatin in intraperitoneal tumors

Distance from the periphery inward to the center	Pt concentration (in ppm):			
	1×4 mg/kg	2×4 mg/kg	3×4 mg/kg	2×10 mg/kg
0.1 mm	9±2	27±4	36±2	77±4
0.5 mm	ND	25±4	ND	73±1
1.0 mm	10±2	24±2	37±3	ND
1.5 mm	ND	20±3	29±4	59±4
2.2 mm	8±3	ND	25±4	49±3
2.5 mm	ND	14±2	ND	ND
3.0 mm	ND	ND	ND	40±2

ND, not determined

were resolved by software developed for analyzing PIXE spectra. This implied that the 28 counts demonstrated in Fig. 1 A are equivalent to 12±4 ppm and that the 11 counts in Fig. 1 B represent 4±2 ppm. The errors are relative, being composed of $\sqrt{}$ B/N, where B is the background area under the peak and N is the area of the peak. Not taken into account were the systematic errors (between 20% and 30%) among fitting procedures in the analyzing software.

Topographic Pt determinations in the kidney

The Pt distribution in the kidney as determined with the PIXE method was compared with ^{195m}Pt distribution. Figure 2 demonstrates an autoradiograph of the kidney of a rat treated i. v. with 5 mg/kg ^{195m}Pt-CDDP. Pt was detectable at higher concentrations in the medulla than in the cortex. Figure 3 demonstrates Pt concentrations in several locations on a micro-beam scan (25-μm proton beam) carried out from the medulla into the cortex of the kidney; the distance between each point was 225 μm. A comparison of both studies revealed the same distribution pattern (Figs. 2, 3). The bar in Fig. 2 from the pelvis into the cortex indicates the line along which PIXE measurements were done. The highest Pt concentration in the medulla was about 25 ppm, whereas Pt concentrations in the cortex declined to about 10 ppm.

Distribution of Pt in i.p. tumors after i.p. administration of CDDP

Distribution studies were conducted in peritoneal tumors to study penetration characteristics of CDDP. Figure 4 shows a three-dimensional plot of the Pt distribution after i. p. treatment with three doses of 4 mg/kg CDDP. This three-dimensional plot, in which the relative Pt concentration (y axis) is plotted as a function of the position in a frozen tissue section (z, x axes), indicates that the highest Pt concentrations occurred at the periphery of the tumor as opposed to the center. This distribution pattern was observed in every tumor, independent of dose and administration schedule, as demonstrated in Table 1. Although the periphery and the center were differently exposed, in both areas the Pt concentrations correlated with the doses delivered (for both the center and the periphery, $r = 0.99$), indicating that superficial cell layers at the periphery of the tumor were exposed to higher drug concentrations than cells in the center.

Penetration depth of CDDP

Figures 5 A and 5 B demonstrate a difference between the distribution of Pt in i. p. tumors in animals treated i. p. and i. v. The i. p.-treated tumors had higher Pt concentrations on the periphery than in the center. This difference was even more pronounced after repeated doses (3×4 mg/kg).

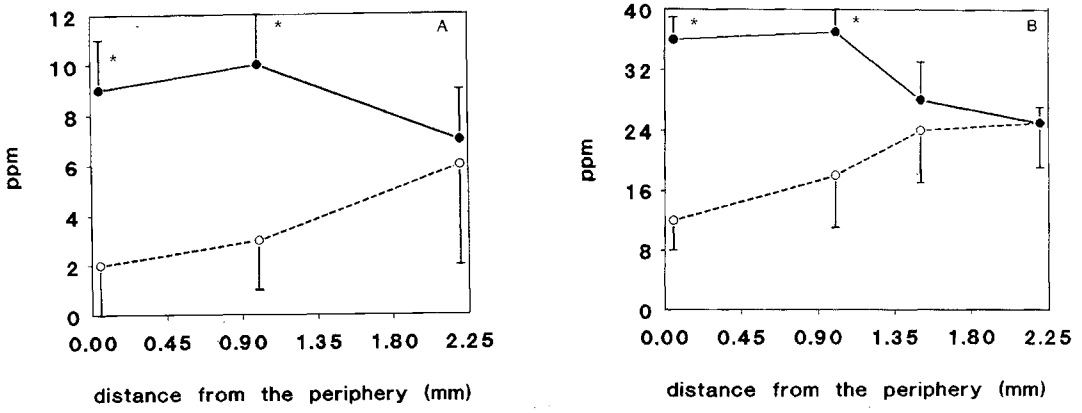

Fig. 5. Pt distribution in intraperitoneal tumors. Tumor-bearing rats (WAG/Rij rat, CC531 tumor) were treated i. v. (O-----O) or i. p (●-----●) with **A** 4 mg/kg and **B** 3×4 mg/kg CDDP. The Pt concentrations were determined by carrying out a line scan from the periphery inward to the center. * Significant difference in Pt concentration after i. p. and i. v. treatments ($P < 0.05$)

No such gradient was found in the i.v.-treated tumors, where Pt concentrations declined in the periphery.

Penetration depth was postulated as the distance to which Pt concentrations were higher after i.p. administration of CDDP than after i.v. treatment. Therefore, Pt concentrations after i.v. administration were subtracted from those after i.p. administration at fixed locations inward from the periphery to the center (Fig. 5). Significant differences in Pt concentrations were demonstrated in the first 1–1.5 mm inward from the periphery. It is clear that for both administration schedules (1×4 mg/kg and 3×4 mg/kg) the concentration advantage lay in the periphery, suggesting that CDDP was delivered to this rim not only by the blood circulation but also by direct penetration of the drug from the peritoneal space.

Discussion

Detection of Pt in small volumes of tissue is a new application of the PIXE technique [8, 9, 23]. In the present study, a micro-beam was used to examine the distribution of Pt in peritoneal tumors. The detection of Pt in small tissue volumes by PIXE was validated by studying the distribution of Pt in the kidney. An autoradiographic study using ^{195m}Pt-labelled CDDP demonstrated the heterogeneous distribution of Pt in the kidney (Fig. 2). Pt accumulated in the medulla and cortex at different concentrations. An identical distribution pattern was demonstrated by PIXE; however, the advantage of this technique was that Pt concentration levels could be quantitatively detected in frozen tissue sections (25×25 μm), whereas with ^{195m}Pt only relative concentrations could be determined. Cisplatin-induced acute renal failure in rats in characterized by a decrease in glomerular filtration rate and urinary concentrating ability. Concomitant with these functional abnormalities, acute pathological changes are seen in the outer medulla of the kidney [2, 5, 6, 11, 32]. These locations were comparable with the area in which the highest Pt concentrations were found (Fig. 3).

Few data are available describing the intratumoral distribution of cytotoxic agents after i.p. drug administration. Ozols et al. [27] evaluated the intensity of intracellular fluorescence of doxorubicin in mouse ovarian tumors, and McVie et al. [24] used PIXE to calculate the Pt content in tumors of patients. In the present paper, we added data on the distribution of Pt within peritoneal tumors after i.v. and i,p. administration of CDDP. It can be concluded, firstly, that penetration of CDDP into peritoneal tumors occurs, and secondly, that a higher exposure to a cytostatic drug leads to a higher drug concentration in the tumor.

In other well-vascularized tissue, such as the liver, linearity has been demonstrated between the i.v. delivered dose of CDDP and the Pt tissue concentration (data not published). This could imply that the center of the tumor, in which a linearity ($r = 0.99$) between dose and concentration exists, is supplied by the blood circulation. Data obtained in previous studies demonstrated that the AUCs measured in plasma were the same after i.p. and i.v. treatment at the same dose of CDDP, demonstrating that the tumor is also likely to be exposed via the blood circulation after i.p. administration [20]. A correlation between dose and concentration also exists on the periphery of the tumor; nevertheless, the concentration is 1 to 2 times higher than in the center of the tumor. An explanation might be that the periphery receives extra, direct drug penetration from the peritoneal cavity. Cisplatin concentrations achieved in the peritoneal cavity were initially 10 to 22 times higher after i.p. injection than the serum levels, which is in agreement with other studies [4, 22, 26]. In another study we have demonstrated that these pharmacological advantages have led to improved survival for i.p.-treated rats vs i.v.-treated animals (manuscript in preparation).

The penetration depth was calculated to be between 1 and 2 mm, corresponding with 50–100 cell layers, depending on the dose schedule. By analyzing experimental data in a rat brain model [16], in which post-microinfusion transport of CDDP has been modelled as a linear diffusion-reaction-permeation process by Morrison and Dedrick [25], the diffusion distance of CDDP was calculated to be 0.8 mm. This difference in diffusion rate can partly be ascribed to difference in tissues, particularly in view of the greater permeability typical of some tumors [18]. Similar analyses, as applied to the brain, should be carried out on tumor tissue to describe a model framework that can be used to assess penetration capacities of cytostatic drugs.

In conclusion, PIXE is a sensitive method for the detection of Pt concentrations in very small volumes of tissue. This technique enabled us to demonstrate the role played by the penetration of CDDP into tumors in i.p. chemotherapy. The comparison of the two routes of administration demonstrated an enormous concentration advantage on the periphery of the tumor for the i.p. route, which we believe is due to direct penetration of CDDP into the tumor. These cisplatin distribution findings, coupled with the fact that i.p. CDDP administration led to clinical responses after the failure of i.v. treatment provide a rationale for further testing of i.p. administration of cytotoxic drugs.

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