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The Long-term Retention of Platinum in Human Tissues Following the Administration of Cisplatin or Carboplatin for Cancer Chemotherapy

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Mass spectrometry has been used to study the distribution and retention of platinum in the tissues of patients following the administration of cisplatin or carboplatin. Blood platinum was measured up to 2 years and renal excretion up to 5 years after treatment. Platinum concentrations in plasma and red cells fell according to a power function, approximately as the inverse square of the time after administration. The concentration in the urine fell more slowly. Necropsy samples were used to examine the distribution of platinum in various human organs up to 17 months after treatment. The highest concentrations were found in the liver, which retained approximately 2% of the dose. Although the results were scattered between patients, there was little loss of platinum after about 1 month. The prolonged retention of platinum may be relevant to long-term toxicity.
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INTRODUCTION

CISPLATIN AND CARBOPLATIN have a wide spectrum of activity against human neoplasms. In particular, the introduction of platinum-based combination chemotherapy has revolutionised the prognosis for patients with non-seminomatous germ cell tumours, of whom a high proportion are now curable with chemotherapy alone [1]. Acute toxicities produced by cisplatin have been well documented and include emesis, ototoxicity, peripheral neuropathy, myelosuppresion and especially, nephrotoxicity [2, 3]. More recently, chronic morbidity associated with cisplatin therapy, including chronic reduction of glomerular filtration rate, peripheral neuropathy, high-tone hearing loss and vasculopathy, including Raynaud's phenomenon, have become apparent [4,5]. Carboplatin leads to less nephrotoxicity, but a greater degree of myelosuppression.

There is an extensive literature describing the early pharmacokinetics, distribution and elimination of cisplatin and carboplatin in humans and animals, but much less is known about the long-term retention of these drugs or their metabolites. It is possible that such retention may be relevant to chronic toxicity.

The limited knowledge of the long-term retention and distribution of platinum has been largely due to the comparative insensitivity of methods of analysis. The availability of a highly sensitive and specific technique, inductively-coupled plasma source mass spectrometry (ICPMS) has allowed us to continue measurements of platinum concentration for a longer period than has previously been possible. We have already described the application of this technique to animal studies [6]; this communication extends its application to measurements on tissues from patients following therapeutic administrations.

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PATIENTS AND METHODS

43 surviving patients, 39 male and 4 female, with an age range of 16–69 years, were studied. Platinum-based drugs were given for the treatment of testicular teratoma (38), ovarian carcinoma (3) or osteosarcoma (2), either alone (3) or in combination with other drugs, vinblastine, etoposide or methotrexate. Patients received cisplatin 100 mg/m^2 over 1 h following prehydration with saline/dextrose, or carboplatin 400 mg/m^2. All patients had normal renal function, as determined by plasma creatinine and renal clearance, prior to chemotherapy. Treatment was repeated to an average of 4 cycles, depending on the chemotherapy protocol used. The average time between the first and final cycle was 2 months. No significant deterioration in renal function was observed. A total dose of approximately 10 mg/kg body weight was usually achieved with cisplatin or 40 mg/kg with carboplatin.

Venous blood was sampled and a 24-h urine collection requested at follow-up clinics, with the aim of covering a wide range of times between administration and sampling.

Post-mortem specimens were obtained from archived material that had been prepared for routine histological examination, from 9 men with teratoma and 7 women with ovarian carcinoma. The wax in which samples were embedded was dissolved out in warm xylene and the tissues rinsed in ethanol. Because of the unknown degree of shrinkage caused by the process of fixation, it was found best to dehydrate the samples, either by freeze-drying or by heat, to constant weight. The original wet weight was then taken to be four times the dry weight, as most organs contain close to 75% water [7].

Sample preparation for ICPMS and the analytical technique used have already been reported [8]. In summary, tissues were digested in hot nitric acid to remove most of the organic material and diluted 10-fold in 1% hydrochloric acid to keep the

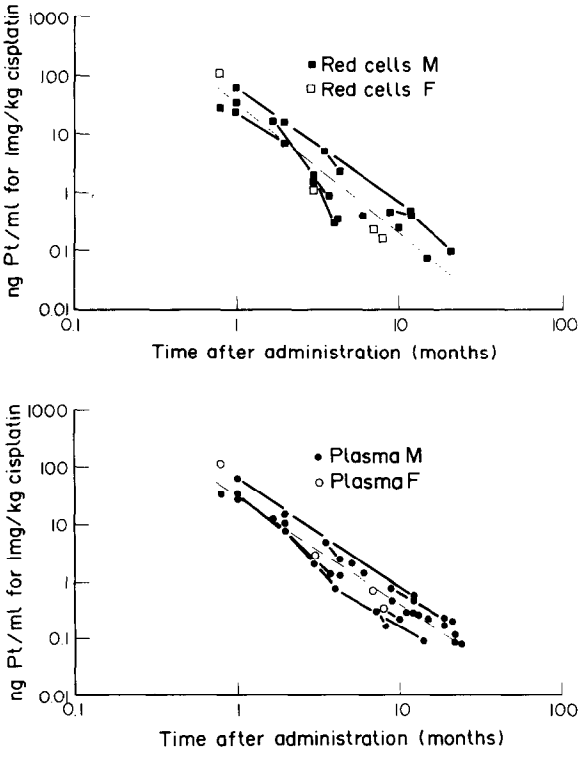

Fig. 1. Concentration of platinum in blood, normalised to a dose of 1 mg/kg body weight of cisplatin, plotted against time between last administration and sampling. Upper: red cells, lower: plasma. M = male, F = female. Full lines join points relating to samples from the same patient. Broken lines are fits from regression analysis.

concentration of salts below 0.2%. The ICPMS apparatus used was a VG Plasmaquad PQ1. The limit of detection for platinum in the original tissue was less than 1 ng/g (parts per billion). The analytical procedure completely disrupts the molecules and no information is given about the chemical form of the platinum in the tissue.

RESULTS

All concentrations of platinum in blood and tissue have been normalised to an administration of the drug at a dose of 1 mg/kg body weight to facilitate comparisons between patients and with results from animal experiments.

The concentrations of platinum in blood are plotted in Fig. 1 on a log–log basis, as an approximately linear relationship was then found to apply. Regression analysis revealed the power function $P = 29.2\ t^{-1.79}$, $r = -0.956$, where P is the platinum concentration in plasma in ng/ml, normalised to a dose of 1 mg/kg of cisplatin and t is the time since the last administration of drug in months. The power function for the red cell concentration was $RC = 29.6t^{-2.14}$, $r = -0.934$. When samples were obtained at intervals from the same patient the points are joined on the graph. The similarity between the slopes of these lines and the mean is a reassuring confirmation of the deductions from the cross-sectional data. There was no significant difference between the regression coefficients for plasma and red cells (t-test).

Urine collections were obtained from 12 subjects treated with cisplatin and 5 with carboplatin. The concentration of platinum in urine was higher than in blood, so that it was still measurable 5 years after cisplatin administration, whereas the limit was about 2 years for plasma. The plasma power function regression for this subset, $P = 28.4t^{-1.93}$, $r = -0.96$, is not significantly different from that from the parent collection. The slope of the urine power function, $U = 54.9t^{-1.17}$, $r = -0.96$, where U is the 24-h excretion of platinum in μg, is significantly less than that for plasma ($P<0.01$, t-test). The plasma power function following carboplatin administration was $P = 3.8t^{-1.48}$, $r = 0.88$ and that for urine was $U = 8.2t^{-0.91}$, $r = -0.99$. Only seven time points were available and the slopes were not significantly different.

Table 1. Urinary excretion of platinum following cisplatin administration

Time since last dose (months)	1	3	10	30
Urinary clearance of Pt from plasma (ml/min)	1.5	3.2	7.4	16.6
% of total dose lost per month	4.4	1.1	0.26	0.07

Urinary platinum clearances were calculated for individuals and groups. The different slopes of the plasma and urine concentration plots meant that the clearance changed with time after drug administration. Some representative mean values for cisplatin are presented in Table 1. The observations for carboplatin covered a shorter range of times, but at 10 months the mean clearance from plasma was 5.5 ml/min, similar to that for cisplatin. Some values of the urinary excretion of platinum expressed as a percentage of the administered dose are also included in Table 1.

Platinum concentrations in post mortem specimens are presented in Table 2. Samples of kidney and liver were obtained from all 16 patients and lung and heart samples from most. Platinum concentrations for these organs are plotted against time in Fig. 2, a logarithmic scale being used for the ordinate in view of the wide range of concentrations. There is considerable variation between subjects and no obvious loss of platinum after 2 months. For this reason, the results in Table 2 are expressed as means of all specimens obtained for a given organ at least 2 months after cisplatin administration. Platinum concentration was much higher in the liver than in any other organ.

In addition to the variability between patients, there is evi-

Table 2. Retention of platinum in organs following cisplatin administration at least 2 months before death

Organ	n	ng Pt/g for 1 mg/kg	Organ weight(g)	% dose
Kidney	12	0.087(0.074)	310	0.06
Liver	12	0.50 (0.25)	1800	2.0
Heart	8	0.062(0.031)	330	0.045
Lungs	11	0.043(0.038)	1000	0.095
Spleen	5	0.068(0.032)	180	0.027
Pancreas	1	0.08	100	0.018
Thyroid	2	0.078	20	0.003
Pituitary	4	0.18 (0.16)	0.6	0.002
Adrenal	3	0.20 (0.12)	14	0.006
Brain	2	0.004	1400	0.012
Prostate	3	0.16 (0.05)	16	0.006
Testes	4	0.16 (0.11)	35	0.012
Uterus	1	0.60	80	0.10
Breast	1	0.30	360	0.24

Mean (1S.D.) for n subjects.

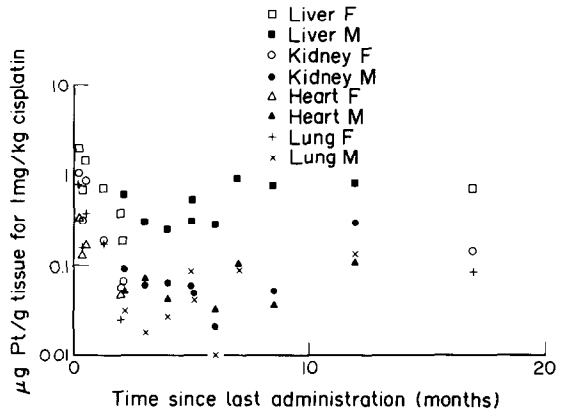

Fig. 2. Concentration of platinum in tissues, normalised to a dose of 1 mg/kg body weight of cisplatin, plotted against time between last administration and death. M = male, F = female.

dence for heterogeneity within organs. For 7 subjects duplicate specimens were measured for one or more organs. When the difference between the two measurements was expressed as a fraction of the mean, values from 0.04 to 1.66 were obtained, with a mean of 0.54. Measurements on post-mortem material taken from control patients who had not been treated with platinum-containing drugs revealed no measurable platinum.

The dependence of platinum concentration on dose and time after administration was examined by linear regression of the data from kidney, liver, heart and lung. There was no significant relationship between concentration and time for any of the organs. Concentration was plotted against total cisplatin dose (without normalisation) for each organ. A relationship between dose and concentration was found for liver, with $P<0.05$ when all data were included and $P<0.01$ when one patient who died only 1 week after treatment was excluded. There were no relationships between dose and concentration for kidney, heart or lung.

The platinum concentrations were multiplied by reference organ weights [7] to obtain some idea of retention as a proportion of the administered dose. For each organ a mean was taken of all the results for times at least 2 months after the last administration. The results are included in Table 2. A mean of 2% of the administered platinum was retained in the liver, more than 20 times that in any other organ.

DISCUSSION

Previous measurements of platinum in blood following cisplatin administration have not proceeded for more than about 1 month. The increased sensitivity offered by ICPMS has allowed the period to be extended to at least 2 years. Pharmacokinetic analysis has previously been based on multi-exponential functions and it has been found that the longer the period considered, the more functions were needed to obtain a fit. For example, when Vermorken *et al.* considered plasma concentrations of platinum up to 5 days after cisplatin administration, three exponential phases were apparent, but extension of data to more than 18 days required a fourth exponential [9]. We have chosen to fit a power function, which characterises the data over the period from at least 1 to 25 months. The implication is that the rate of loss of platinum from plasma decreases continually and that a larger number of exponential functions would be required the longer the study proceeded.

There have been fewer reports of platinum concentration in red cells than in plasma, but there are suggestions that over a period of a few weeks the red cell content is lower but decreasing more slowly [9,10]. We find that the concentration was approximately the same at 1 month, but at 1 year the red cell concentration was lower.

The pattern of changes in blood platinum over the time scale considered here may be compared with those in experimental animals dosed with cisplatin. We found that platinum concentration in rat plasma could also be well fitted by a power function over the period of 1–12 weeks [6]. The exponent of the equation was not much different from that in humans, but the constant was much lower. Other measurements of blood concentrations soon after cisplatin administration show that the differences of normalised concentration persist at the earliest time of measurement, 1 h after administration.

Measurements of platinum in blood were also made in pigs, for 1 year after administration [6]. The blood concentrations of platinum are compared in the three species in Fig. 3, using a log–linear scale in the interests of familiarity. It can be seen that the normalised levels are similar in pigs and humans for about 3 months, but thereafter the values for humans fall more slowly.

Most measurements of the urinary output of platinum have also not previously extended beyond a week, by which time about half of the dose of cisplatin has been excreted [11,12, 13]. Dominici *et al.* [14] did plot measurements of platinum concentration for a month, but did not indicate urine volumes, so that 24-h outputs cannot be estimated. Very few estimates of the renal clearance of platinum from plasma have been made. Smith and Taylor [11] calculated clearances of 1.3 and 1.8 ml/min in 2 patients during the first 3 days, similar to our values at 1 month. Even though the renal clearances increased with time, the values remained relatively low, as expected from a high degree of protein binding of platinum.

Both blood and urine concentrations of platinum were lower over the period of our measurements for carboplatin than for cisplatin administrations, in accordance with the greater early excretion of the former [15].

Few studies have been made of the retention of platinum in tissues as determined from autopsy samples. An early report from Hill *et al.*described results from 2 subjects who died 2 and 25 days after therapy [16]. Stewart *et al.* [17,18], using material from 12 patients up to 8 months after treatment, obtained similar results to ours and also found the highest concentration of platinum in the liver. Stewart *et al.* [18] found that a relationship existed between dose of cisplatin and platinum concentration for the liver, but not for the kidney. Our results are in agreement.

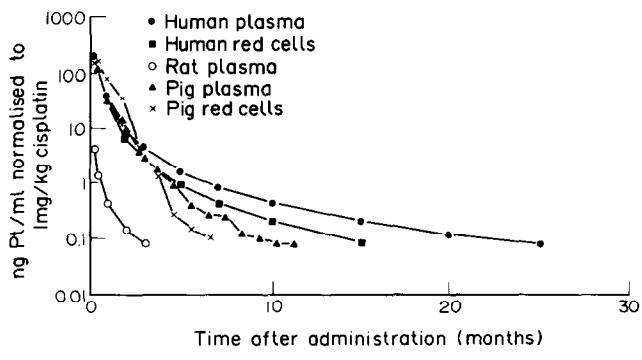

Fig. 3. Mean concentrations of platinum in blood, normalised to a dose of 1 mg/kg body weight of cisplatin, plotted against time after last administration, for three species.

The lack of a dose dependence of platinum concentration is surprising, but its confirmation in our study suggests that this may represent a saturation phenomenon.

Stewart *et al.* found an inverse correlation between concentration of platinum in the renal cortex and time from last treatment but no such relationship for the liver [18]. We find no significant dependence on time for any organ, which is at first sight also surprising. However, there is considerable spread of results between patients. In addition, the measurements of urinary platinum in living patients suggest that there would be a relatively small loss of platinum from the body over the period considered. A small amount of platinum is excreted in bile [19, 20], but it is considered that the major excretion route is via the kidneys. Table 1 shows that from 1 month onwards, only a small proportion of the administered dose is lost from the body and the scatter of results would completely mask such a slope.

The retention of platinum in human tissues may be compared with that in experimental animals. We obtained data from rats and pigs over similar time scales to those for the autopsy samples [6]. With both species there was much less scatter of results, as would be expected from prospective experiments with well-matched animals and protocols. The relationship between platinum concentration and time approximated to an inverse power function, so that the rate of loss of platinum diminished continuously. The loss from tissues was much slower than from blood.

In rats, studied for 3 months, the highest concentrations of platinum were in the kidney and spleen. Pigs were more like humans, with the highest concentrations in the liver, at times up to a year. However, when organ mass was taken into account, in both species the greatest amount of platinum was retained in the major tissues muscle, skin and bone. Total body retention was measured directly in rats, being nearly 4% of the dose at 3 months, and estimated in pigs, being more than 5% at 11 months. In all organs for which measurements are available, the platinum content in human tissue presented in Table 2 was higher than the corresponding values for pigs at 11 months after cisplatin administration, suggesting that the total retention of platinum in humans might well exceed 5% over a substantial period.

Our measurements show that, of the organs examined, the liver had much the highest concentration. Although hepatotoxicity is not considered to be a serious problem in patients treated with cisplatin, proliferative lesions have been observed in mice 18 months after cisplatin administration [21]. In any case, the liver represents a reservoir of platinum. The animal experiments suggest that more substantial reservoirs exist in the skin, muscle and bone. Platinum is a potentially toxic element in many forms and our observations should contribute towards an understanding of long-term toxicity.

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