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Alterations in plasma pharmacokinetics of cisplatin in tumor-bearing rats

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Summary. Rats were inoculated s.c. with the Walker 256 solid carcinosarcoma, and when tumors reached a weight of approximately 2–3 g, pharmacokinetics, tissue distribution, and urinary excretion of ^{195m}Pt-labelled cisplatin were studied. Cisplatin was given i.v., blood was sampled through arterial cannulae, and data were fitted to a three-compartment model. Distribution half-times were prolonged two- to threefold in tumor-bearing animals, although there was no change in elimination half-time. Initial and steady-state volumes of distribution were also increased in tumor-bearing animals. There was no change in AUC, urinary excretion, tissue distribution, or plasma protein binding. The results indicate that a solid tumor represents an additional compartment for distribution of cisplatin and alters the rate at which cisplatin is distributed from the plasma.

Introduction

Preclinical toxicologic and pharmacologic effects of anticancer drugs are usually determined in normal, healthy adult animals. However, antitumor efficacy is determined in tumor-bearing adult animals. What has only recently been appreciated is that the presence of tumor may alter the toxicity and/or the pharmacologic efficacy of drugs. The presence of a large tumor can be expected to alter various nutritional or physiologic parameters in animals, and these changes may be responsible for observed differences in drug toxicity between control and tumor-bearing animals [1, 7]. In addition, tumors have the potential to disrupt normal drug pharmacokinetics and distribution. However, only a few previous reports have addressed this important question. Generally, distribution of a drug into a tumor or other bulky mass would be expected to increase clearance half-times and the volume of distribution. An early and preliminary investigation into the effect of tumor presence on the pharmacokinetics of the antineoplastic drug cisplatin showed an increased distribution half-time and a dramatically decreased elimination half-time, but with little drug present in the tumor [15]. These intriguing preliminary results prompted us to investigate in detail the effect of tumor presence on the pharmacokinetics and distribution of cisplatin in rats carrying the solid Walker 256 carcinosarcoma tumor.

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Methods and materials

Cis-dichlorodiammineplatinum(II) (cisplatin) was obtained from the Division of Cancer Treatment, National Cancer Institute, and was dissolved on 0.9% NaCl at 1 mg/ml immediately before use. Cisplatin labelled with ^{195m}Pt (specific activity = 0.46 mCi/mg) was purchased from Oak Ridge National Laboratory (Oak Ridge, Tenn, USA) and was used without further purification. Walker 256 carcinosarcoma was originally obtained from Dr. A. Bogden (Mason Research Institute, Worcester, Mass, USA) and was maintained by serial s.c. transplantation into 50- to 60-day-old female Sprague Dawley rats (Sprague Dawley, Madison, Wis, USA).

Female Sprague Dawley rats (50–60 days of age) weighing 180–200 g received an s.c. implant of Walker 256 tumor fragments (approximately 300 mg) in the flank. Tumor sizes were estimated 7 days later by two-dimensional measurements using calipers, and abnormally large or small tumors were culled. The tumors selected for the study were approximately $2 \times 2 \times 2$ cm^3, weighed 1.5–2.9 g, and represented 1%–2% of the total body weight. All subsequent procedures were similar for tumor-bearing rats and for non-tumor-bearing, sex- and age-matched controls. From each group, 12 rats were anesthetized with i.p. pentobarbital (35 mg/kg) and polyethylene cannulae (PE-50) were implanted into the femoral vein and artery. The incision was closed with sutures. When the animals had fully awakened from the anesthetic, 4 mg/kg cisplatin containing ^{195m}Pt was injected into the venous cannula. The quantity of radioactivity per rat varied, depending on how long after injection the animals were to be kept. Thus, rats kept for 4 days received 100 μCi and those kept for only 10 min received 20 μCi. Blood samples (200 μl) were drawn from the arterial cannula into heparinized syringes at times ranging from 2 to 480 min after drug injection. At selected times between 2 and 180 min, 1 ml blood was drawn to allow for separation of a protein-free ultrafiltrate (Centrifree filtration membranes; Amicon Corp., Danvers, Mass, USA).

Three cannulated rats were killed with pentobarbital overdose through the venous cannula at 10 min, 60 min, 4 h, and 8 h after dosing, and the following tissues were excised and analyzed for total radioactivity: brain, thymus, heart, lung, liver, spleen, kidney, muscle, perirenal fat, skin, duodenum, ovary, and mesenteric lymph nodes. Plasma levels of radioactivity at times between 8 h and 4 days and the urinary excretion of radioactivity were ob-

tained by lightly anesthetizing six each of the control and tumor-bearing rats with i.p. pentobarbital (25 mg/kg) and then injecting each rat through the tail vein with 4 mg/kg cisplatin containing ^{195m}Pt. These rats then placed into stainless steel metabolic cages. Urine was collected and volume determined at 30, 60, 90, and 120 min, at 4, 8, and 12 h, and then daily through day 4. Three rats from each group were killed at 24 h and three at 96 h after treatment. Tissues and fluids were analyzed as above. Radioactivity was determined in a Packard Gamma counter set at 80% gain with discriminators at 200 and 700. A sample of each radioactive drug solution was counted at the time of treatment and then again with each separate set of tissues or fluids to allow correction for radioactive decay. Plasma radioactivity data were corrected for decay and all data were pooled for pharmacokinetic calculations. Plasma decay of radioactivity over the 96-h time course was fit to a three-compartment open model, and distribution and elimination half-times were calculated using a least square, nonlinear method on MLAB [8]. Values for pharmacokinetic parameters were derived by MLAB using standard equations. All differences between control and tumor-bearing animals were evaluated using Student's t-test at $P \leq 0.05$.

Results

Pharmacokinetic parameters were different in tumor-bearing and control rats, as shown in Fig. 1. The half-times for distribution were longer in tumor-bearing than in control rats, but the terminal elimination half-time was the same in both groups. Other parameters were also different in the tumor-bearing rats. The volume of distribution at steady state and the initial volume of distribution were larger in the tumor-bearing rats than in controls. Interestingly, clearance (Cl) was the same in both groups. The values obtained for these parameters in the non-tumor-bearing controls are consistent with previous measurements from non-tumor-bearing male rats.

Tables 1 and 2 show the concentration of ^{195m}Pt in tissues at various times after the injection of ^{195m}Pt-labelled cisplatin. A comparison of the two tables shows little difference in distribution pattern or retention of radioactivity between the two treatment groups. In addition, ^{195m}Pt was found in tumor tissue at all times studied and at quantities up to 1.5% of the dose given. Excretion of radioactivity was also similar in the two groups. Tumor weights were 1.6 ± 0.3 g in the 8-h group, 2.9 ± 0.3 g in the 60-min group, and 1.3 ± 0.4 g in the 4-day group. There were no differences in plasma protein binding of platinum between the two groups at times between 2 and 240 min after drug injection (data not presented).

Discussion

The presence of a relatively small, solid tumor resulted in a significantly slower distribution of cisplatin-derived ra-

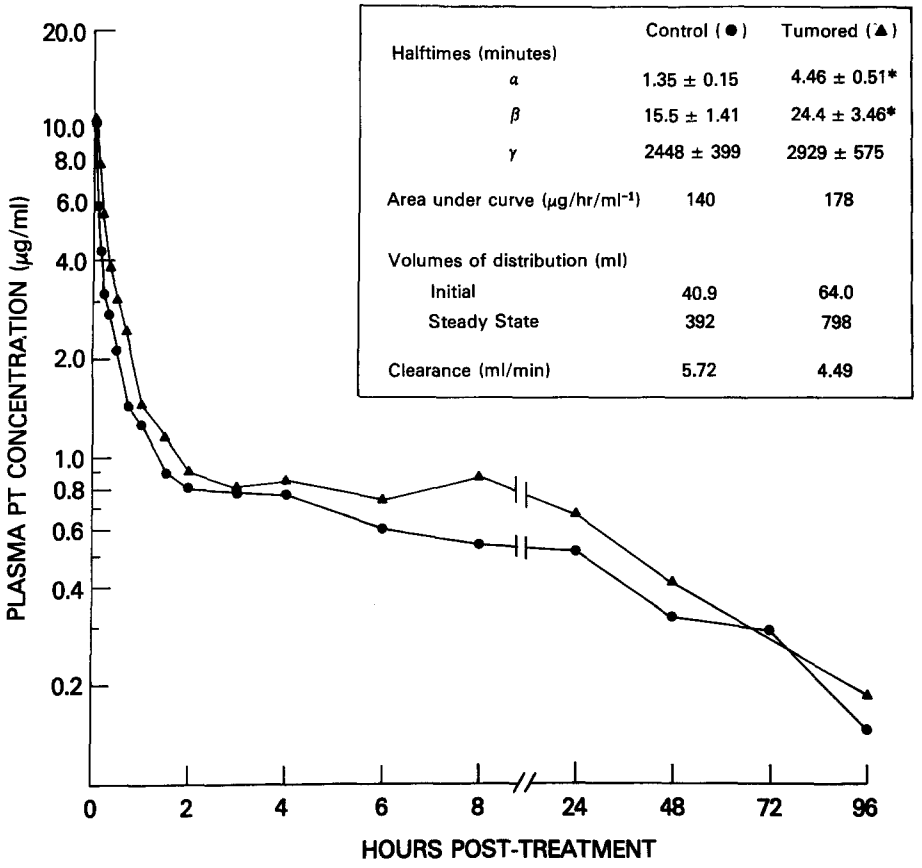

Fig. 1. Plasma concentration of platinum in tumored and nontumored rats following intravenous administration of ^{195m}Pt-labelled cisplatin. Each point is the mean of 6–8 rats (0–8 h) or 3–6 rats (24–96 h). Insert shows calculated and derived pharmacokinetic parameters. Asterisks indicate differences between control and tumored data at $P = 0.05$

Table 1. Concentration of ^{195m}pt (μg/gm × 10^{-3}) in tissues of rats treated with 4 mg/kg ^{195m}pt-containing cisplatin

Tissue	Time					
	10 min	60 min	4 h	8 h	24 h	96 h
	(n = 3)	(n = 3)	(n = 4)	(n = 4)	(n = 3)	(n = 3)
Lung	3.66±0.41	2.10±0.13	2.26±0.19	1.82±0.15	1.44±0.23	0.74±0.07
Heart	1.52±0.28	0.86±0.04	0.62±0.05	0.61±0.06	0.53±0.08	0.29±0.02
Liver	4.19±0.75	3.92±0.28	4.00±0.22	3.65±0.32	2.70±0.19	1.31±0.13
Kidney	13.4 ±1.7	10.8 ±0.5	11.6 ±1.0	10.6 ±1.5	10.0 ±0.9	7.16±0.32
Spleen	1.82±0.11	1.67±0.06	1.62±0.17	1.51±0.14	1.56±0.10	1.53±0.19
Colon	2.96±0.41	1.87±0.27	1.76±0.18	1.43±0.16	1.17±0.09	0.49±0.06
Duodenum	3.97±0.80	2.66±0.37	2.15±0.23	1.91±0.28	0.98±0.10	0.32±0.04
Skin	3.69±0.09	2.37±0.21	2.20±0.41	1.98±0.15	1.67±0.31	0.92±0.08
Muscle	ND	ND	0.34±0.01	ND	0.34±0.13	0.18±0.03
Plasma	4.76±0.40	1.48±0.08	0.77±0.06	0.55±0.16		
Urine[a]	11.1 ±1.6	33.0 ±8.8	ND	51.3 ±8.7	63.1 ±6.2	67.2 ±7.3

[a] Cumulative percentage of dose
ND, Not determined

Table 2. Concentration of ^{195m}pt (μg/g) in tissues of tumored rats treated with 4 μg/kg ^{195m}pt-containing cisplatin

Tissue	Time					
	10 min	60 min	4 h	8 h	1 day	4 day
	(n = 4)	(n = 4)	(n = 2)	(n = 5)	(n = 3)	(n = 3)
Lung	4.53±0.10	1.77±0.48	1.78±0.31	1.56±0.21	1.80±0.16	0.81±0.01
Heart	2.13±0.16	0.94±0.17	0.75±0.05	0.77±0.04	0.71±0.11	0.36±0.04
Liver	5.44±0.48	4.47±0.39	3.45±0.45	3.85±0.63	3.83±0.17	2.31±0.16
Kidney	20.7 ±0.8	10.8 ±5.2	8.82±0.43	9.63±1.72	10.6 ±2.3	9.68±1.37
Spleen	2.51±0.41	1.66±0.18	0.95±0.31	1.36±0.50	2.46±0.22	2.54±0.14
Colon	3.52±0.48	2.08±0.60	1.85±0.24	1.50±0.14	1.49±0.31	0.73±0.12
Duodenum	4.47±0.31	2.86±0.50	1.88±0.14	2.10±0.24	1.51±0.14	0.53±0.11
Skin	5.38±0.46	3.65±0.31	2.71±0.25	3.50±0.29	2.91±0.38	1.15±0.08
Muscle	1.46±0.16	0.69±0.08	0.46±0.01	0.53±0.04	0.49±0.07	0.34±0.03
Tumor	8.32±0.24	3.18±0.59	2.98±0.07	2.14±0.53	5.13±0.97	2.93±0.52
Plasma	5.84±0.94	1.60±0.53	1.25±0.07	0.87±0.15	0.79±0.10	0.18±0.02
Urine[a]	16.6 ±7.0	34.9 ±8.8	49.1 ±6.1	53.2 ±4.9	56.4 ±2.8	63.6 ±4.4

[a] Cumulative percentage of dose

dioactivity from the blood into tissue. These changes in alpha and beta half-times are consistent with changes observed in a preliminary study of cisplatin kinetics in tumor-bearing animals [15] as well as with the effect of tumors on the pharmacokinetics of anticancer drugs in patients [4]. In the present study, there was no change in elimination half-time, however, in the presence of a tumor. This is also consistent with the effects of tumors on the pharmacokinetics of other anticancer drugs in tumor-bearing animals [11] but is in contrast with preliminary results reported for cisplatin in such animals [15]. The lack of effect on elimination half-time, however, is reflected by data in Tables 1 and 2, which show no significant difference in the rate or extent of renal excretion of radioactivity between the control and tumor-bearing groups. Furthermore, there were no differences in total area under the plasma concentration time curves. This is consistent with findings involving other anticancer drugs in tumor-bearing animals [2]. The similar AUCs can be explained because the largest portion of the AUC is contributed from the third compartment of a three-compartment model, and the half-times of this compartment were equal in both groups of this study.

Tumor presence resulted in larger initial and steady-state volumes of distribution. This was not entirely unexpected because the tumor, although small, would represent an additional compartment into which the drug could distribute. Also, Donelli et al. [6] have reported a "markedly" increased volume of distribution of 6-mercaptopurine in tumor-bearing animals. The increased volume of distribution is reflected by the presence of relatively high concentrations of platinum in the tumor at all times studied. The high concentration of platinum in the tumor contrasts with that of other studies, which show lower concentrations of platinum in tumor tissue, but this discrepancy may be due to our substantially higher dose or to a different tumor type. The localization of platinum in tumor tissue is variable and apparently dependent upon tumor type, with a small uptake reported for sarcoma 180 in mice [3, 9] and relatively little uptake reported into human osteogenic sarcoma and fibrosarcoma [3]. Early whole-body counting of

patients who received ^{195m}Pt-labelled cisplatin has recorded no evidence of platinum in ovarian cancer or in malignant melanoma [12], although no information was provided about the time between drug injection and performance of the scans. Other studies, however, have reported easily measurable concentrations of platinum in brain tumors at relatively prolonged times after patients were given cisplatin [5, 13].

The excretion of platinum from tumor-bearing rats was the same as that from control rats and was within the expected range of platinum excretion for this species. The similar plasma protein binding by the two treatment groups suggests, among other things, that the tumor is not releasing any high-molecular-weight, platinum-binding substances into the circulation.

There were no differences in the tissue distribution of platinum in the presence of tumors, which is consistent with results for other anticancer drugs [10]. Perhaps if the tumor had been allowed to grow to a larger size, the distributional differences might have been greater. Substantial differences have been observed in the distribution of the nitrosourea BCNU in the livers of tumor-bearing rats, but these have been attributed to the extensive hepatic metabolism of this drug [2], which is similar to that which has been shown for tumor-associated differences in the metabolic rate of pentobarbital [14]. No data are available, however, for anticancer drugs that are not metabolized, such as cisplatin.

In conclusion, tumors provided an additional compartment that resulted in increased distribution half-times and an increased volume of distribution, both initially and at steady state. However, there was no change in renal clearance, organ distribution, or urinary excretion between tumor-bearing and control animals. It is unlikely that tumor presence would alter toxicity or other distribution-dependent drug parameters.

References

1. Barcellona PS, Campana A, Rossi V, Corradino C, Silvestrini B (1984) Comparative study of acute toxicity of drugs used during anticancer therapy in healthy and tumor-bearing mice. Arch Toxicol (Suppl) 7: 90
2. Bartosek I, Russo RG, Cattaneo MT (1984) Pharmacokinetics of nitrosoureas: levels of 1,3-bis-(2-chloroethyl)-1-nitrosourea (BCNU) in organs of normal and Walker 256/B carcinoma bearing rats after i.v. bolus. Tumori 70: 491
3. Benard P, Desplanches D, Macquet JP, Simon J (1983) Whole-body autoradiographic study of the distribution of ^{195m}Pt in healthy and tumor-bearing mice treated with cisplatin. Cancer Treat Rep 67: 457
4. Benjamin RS (1974) Pharmacokinetics of adriamycin (NSC-123127) in patients with sarcomas. Cancer Chemother Rep (part 1) 58: 271
5. Bonnem EM, Litterst CL, Smith FP (1982) Platinum concentrations in human glioblastoma multiforme following the use of cisplatinum. Cancer Treat Rep 66: 1661
6. Donelli MG, Colombo T, Forgione A, Garattini S (1972) Distribution of 6-mercaptopurine in tumor-bearing animals. Pharmacology 8: 311
7. Harrison SD, Giles HD, Denine EP (1980) Antitumor drug toxicity in tumor-free and tumor-bearing mice. Cancer Chemother Pharmacol 4: 199
8. Knott GD (1981) MLAB: A mathematical modeling tool. Comput Programs Biomed Res 10 (3): 271
9. Lange RC, Spencer RP, Harder HC (1973) The antitumor agent cis-Pt $(NH_3)_2Cl_2$: distribution studies and dose calculations for ^{193m}Pt and ^{195m}Pt. J Nucl Med 14: 191
10. Lowenthal IS, Parker LM, Greenblatt DJ, Brown BL, Samy TSA (1979) Pharmacokinetic analysis of neocarzinostatin in normal and tumor-bearing rodents. Cancer Res 39: 1547
11. Nooter K, Sonneveld P, Martens A (1985) Differences in the pharmacokinetics of daunomycin in normal and leukemic rats. Cancer Res 45: 4020
12. Smith PHS, Taylor DM (1974) Distribution and retention of the antitumor agent ^{195m}Pt-cisdichlorodiammine platinum (II) in man. J Nucl Med 15: 342
13. Stewart DJ, Leavans M, Maor M, Feun L, Luna M, Bonura J, Caprioli R, Loo TL, Benjamin RS (1982) Human central nervous system distribution of cis-diamminedichloroplatinum and use as a radiosensitizer in malignant brain tumors. Cancer Res 42: 2472
14. Villa P, Guaitani A, Bartosek I (1978) Differences in pentobarbital disappearance rate in rats bearing two lines of Walker carcinosarcoma 256. Biochem Pharmacol 27: 811
15. Wolf W, Manaka R (1977) Synthesis and distribution of ^{195m}Pt cisdichlorodiammineplatinum (II). J Clin Hematol Oncol 7: 79–95

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