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Two Types of Tumour Sensitivity Test Compared for Platinum Derivatives*

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Abstract—*For early passages of* 15 *human tumours grown in nude mice two types of test for prediction of sensitivity to cytostatic drugs were carried out for four platinum compounds. The subrenal capsule assay of Bogden was not found to be useful for these early passages, since the response of duplicate tests was not very different from randomly distributed results. The clonogenic assay as described by Hamburger and Salmon gave reproducible results on the drug sensitivity of those tumour cells that grew colonies* in vitro. *However, in a limited number of cases irregular colony growth occurred. Lower drug concentrations were apparently more effective in killing cells than higher concentrations. From the replicate test results it became clear that such results are not a reliable indicator of drug sensitivity. Furthermore, the critical drug concentration for optimal testing of drug effectiveness is probably not well represented by a uniform relation to peak plasma level.*

INTRODUCTION

IN THE last decade, tumour sensitivity tests have received increased attention as potential means of supporting antitumour chemotherapy and more specifically as tools to make it possible to avoid unnecessary exposure of the patient to treatment with ineffective but toxic cytostatic agents. It is difficult to compare the validity of these methods on the basis of their contribution to the effectiveness of treatment [1]. For that reason, we have made a comparative study of the reproducibility of results of sensitivity tests carried out on four platinum compounds. A comparison was made of two systems that have been used to predict drug sensitivity; the subrenal capsule assay described by Bogden *et al.* [2] and the clonogenic assay described by Hamburger and Salmon and their colleagues [3, 4]. A comparison between the precursor incorporation test, a monolayer culture assay and the clonogenic assay has been made by Wilson *et al.* previously [5] on two long established cell lines. It seemed useful to study duplicate results of different tests carried out on early passages of human tumours in nude mice in order to approach as much as possible the situation of routine individual tumour sensitivity tests. We report here the results obtained in a comparison of this type.

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MATERIALS AND METHODS

Human tumours

Samples of operation specimens of human tumours were implanted in nude mice and the first or second passage was stored in liquid nitrogen as a coarse brei in Hanks' balanced salt solution (BSS) supplemented with 5% calf serum and a final concentration of 10% dimethylsulfoxide. Sensitivity tests were performed usually from the second to the eighth passage; one tumour (Lu 4) was studied up to passage 11. The frequency of successful growth of these early passage tumours in nude mice was not high; many were not fully adapted to growth as xenografts. Tumours of the ovary, the colon and the lung were predominantly studied, but occasionally other tumours were included: a yolk sac tumour and an adrenal tumour. No data were available on clinical response of these tumours to single cytostatic drugs.

Platinum compounds

The four platinum-containing cytostatic compounds were: (a) cisplatin (*cis*-diamminedichloroplatinum (II); NSC 119875); (b) iproplatin, CHIP (*cis*-dichlorotransdihydroxybis(isopropylamine)platinum (IV); NSC 256927); (c) carboplatin, CBDCA (*cis*-diammine-1,1-cyclobutanedicarboxylate platinum (II); NSC

241240); and, (d) spiroplatin, TNO-6 (*cis*-1,1-di(aminomethyl)cyclohexaneplatinum (II) sulphate; NSC 311056). Cisplatin and CBDCA were dissolved in 0.9% NaCl solution, TNO-6 and CHIP in 5% glucose solution.

The subrenal capsule assay

The procedure of Bogden *et al.* [2] for normal mice was modified; mice were irradiated before grafting [6]. Male 6–8-week-old (C57BL/Rij × CBA/Rij)F_1 hybrid mice were exposed to 4 Gy whole-body irradiation from a ^{137}Cs source within 4 hr before tumour implantation. Tumours were aseptically collected from nude donor mice and from suitable portions of the tumour cubes of 1 × 1 × 1 mm^3 were cut. The irradiated recipient mice were anaesthetized with Avertine,® 7.5 mg i.p. After shaving and preparation of the skin with alcohol (70%) the skin was incised over one of the kidneys, the muscle layer was divided and the kidney was exteriorized. The capsule was incised and with a trocar a single cube of tumour was placed under the capsule. The presenting tumour surface area was then measured in two perpendicular dimensions with the aid of a microscope grating marked in 0.1 mm. The kidney was returned to the abdominal cavity and the wound was closed with staples. Six days later the wound was reopened and the tumour diameters were measured again. The results of treatment were expressed as change in mean tumour diameter (delta diameter, or dd) for each tumour graft:

$$dd = \frac{\text{length} + \text{width (day 6)}}{2} - \frac{\text{length} + \text{width (day 0)}}{2}.$$

In addition to 8–10 untreated control mice, five mice were used for each drug; they received two i.v. injections of two-thirds of the LD_{50} dose, on days 1 and 5 after grafting. The specific quantities used were: cisplatin, 8 mg/kg; CHIP, 30 mg/kg; CBDCA, 90 mg/kg, and TNO-6, 4.5 mg/kg for each dose.

The clonogenic assay

Pilot studies on the culture of human tumour cells led us to the introduction of a number of modifications in the method of Hamburger and Salmon [4]. The addition of 0.006 ml of rat (Brown Norway BN/BI) erythrocytes to the lower layer [7] nearly doubled colony yield. DEAE–dextran was not added to the bottom layer [8] and the concentration of $CaCl_2$ in the top layer was halved.

For culturing ovarian tumours, 2-mercaptoethanol was omitted from the top layer [7]. For the preparation of cell suspensions the solid tumours were minced with scissors and incubated for 2 hr at 37°C in complete medium containing collagenase (1168 IU/ml, Sigma type 1A) and DNase (62 IU/ml, Sigma D-1001) in an atmosphere of 5% CO_2 in air. Suspensions were centrifuged at 150 ***g*** for 10 min and washed twice in modified McCoy's medium (with 10% fetal calf serum, penicillin 200 IU/ml and streptomycin 200 μg/ml final concentration). Viability of the cells was determined with eosin during counting. Pilot tests had shown that 10^5 cells per dish yielded the highest plating efficiency, so this was used in all tests. The platinum compounds were prepared in concentrated sterile solutions and stored at −20°C for individual tests. Each drug was tested at three concentration levels. In addition, the effects on colony formation were studied both after 1-hr exposure to the drug and after continued exposure during the 2-week culture period. For the latter procedure agents were added at the time of plating, but no attempts were made to replace inactivated drug.

All assays at each drug concentration were performed in triplicate. On day 1 some dishes were checked for the presence of clumps that might falsely simulate colony growth. A correction for clump counts was made. The cultures were kept at 37°C with 5% CO_2 in air for 2 weeks. The colonies containing 40 or more cells were counted at ×10 magnification. Occasionally use was made of INT staining to verify viability of the colonies. At least 30 colonies per control dish were required to assure an adequate evaluation of drug effects.

In addition, the effect of the platinum compounds was determined against mouse leukaemia L1210 in a similar assay as used for the human tumours. L1210 cells from a suspension culture were exposed to the drugs for 1 hr and then plated in 35-mm Petri dishes or cultured for 8 days in the presence of the drugs. As described earlier [9] cells were grown in a single layer of soft agar. For each compound 6–8 twofold serial dilutions were used to determine the concentration that caused 75% inhibition of colony formation.

RESULTS

The subrenal capsule assay

Tests were considered inadequate for evaluation if control tumour change in mean graft diameter was less than −0.05 mm [2], i.e. rather than growing, control tumours shrank more than 0.05 mm. This occurred in 7/36 tests. In a number of tests not all tumours were available for sixth-day reading of results (due to toxic death of mice or loss of the graft from the renal capsule). In Table 1 the

averaged data of at least seven control mice and at least four animals per drug treatment are shown. In 29 tests the criteria for adequate control growth were met and from 12 tumours duplicate or triplicate test results were obtained. Results were considered positive if there was a significant difference ($P < 0.05$) in change of graft size from day 0 to day 6 between treated mice and controls. Positive tests were noted in 12/29 grafts exposed to cisplatin, 11/28 exposed to CHIP, 12/28 exposed to CBDCA and 8/27 exposed to TNO-6. Comparison of the duplicate test results revealed poor correlation. Using the criterion of significance ($P < 0.05$) of the difference between treated and control results, agreement of consecutive duplicate tests was noted in 35/60 drug data. A random distribution of this fraction of positive responses would lead to agreement in 32/60 tests.

The clonogenic assay

Of the 41 tests performed, only 23 resulted in sufficient tumour growth, 12 demonstrated insufficient or no growth and seven cultures were infected. In addition, for some tests the tumour sample (at least 1 g) yielded insufficient cell numbers to carry out both a 1-hr incubation and a

Table 1. *Response of tumours in the subrenal capsule assay*

Tumour	Control growth*	*P* value for difference with control			
		Cisplatin	CHIP	CBDCA	TNO-6
Ovarian tumours					
OvP	0.57	0.69	0.33	0.58	0.84
OvP	0.52	0.019	0.039	0.98	0.62
OvL	0.16	0.059	0.12	0.75	0.23
OvL	0.01	0.90	0.19	0.83	0.20
OvL	0.01	0.026	1.0	0.56	0.52
OvS	0.18	0.019	0.59	0.22	0.075
OvS	0.10	0.006	0.013	0.021	0.015
OvC	0.06	0.036	0.006	0.035	(n.a.)
OvC	0.16	0.070	0.19	0.12	0.43
OvT	0.52	0.031	0.012	0.012	0.12
Colon tumours					
ColH	0.37	0.012	0.0021	0.026	0.011
ColH	0.33	0.031	0.37	0.27	0.024
ColH	0.08	0.39	0.001	0.017	0.027
ColF	0.16	0.004	0.16	(n.a.)	0.14
ColF	0.36	0.075	0.025	0.26	0.57
ColK	0.05	0.27	0.26	0.17	0.33
ColK	0.03	0.052	0.11	0.19	0.017
Lung tumours					
Lu1	−0.02	0.076	n.a.	0.078	n.a.
Lu1	−0.03	0.23	0.19	0.065	0.095
Lu1	−0.02	0.056	0.051	0.0075	0.097
Lu2	0.54	0.052	0.012	0.035	0.011
Lu2	−0.01	0.70	0.50	0.30	0.99
Lu3	0.38	0.11	0.51	0.018	0.095
Lu3	−0.03	0.37	0.32	(0.008)	0.62
Lu3	0.04	0.072	0.067	0.20	0.17
Lu4	0.44	0.0017	0.026	0.025	0.044
Lu4	0.66	0.00025	0.019	0.00002	0.011
Yolk sac tumour					
Ys1	0.31	0.62	(0.079)	0.078	0.062
Ys1	0.47	0.0007	0.0034	0.0026	(0.13)

n.a. = not available, usually less than four samples remaining at end of test. *P* values below 0.05 are underlined. Seven tests did not meet the criteria for minimal control growth of −0.05 mm.

*Change in average diameter (mm).

continuous incubation, so results are presented for 19 1-hr exposure tests and for 21 continuous exposure tests (Tables 2 and 3). For seven tumours duplicate or triplicate results were available to permit evaluation of reproducibility. For statistical analysis, triplicate tests were split into two sequentially performed duplicate results. Tests were considered positive if clone survival in the treated dishes was 30% or less compared to control dishes. After 1-hr exposure sequential duplicate tests for individual drugs agreed in 19 cases, showed a one (tenfold)-dilution difference in 11 cases and a two-dilution difference in three cases. For the continuous drug exposure these figures are 17, 12 and 11. The latter procedure appears less dependable since it shows more frequently erratic results such as lower drug concentrations appearing more effective in killing cells than higher concentrations. This may be the consequence of the fact that tests in which cell yield was insufficient to perform both the 1-hr and the continuous exposure tests were usually performed exclusively with continuous exposure and it is among these tests that the frequency of erratic results was higher. For the 1-hr test the agreement between duplicates was also analysed separately at each concentration level. At the highest concentration tested, a correlation coefficient between duplicate test results of 0.45 with $P = 0.038$ was calculated and similar results for the next lower concentration.

If we tabulate the results against exposure (Table 4), it appears that the 1-hr exposure data

Table 2. Growth of human tumour cell colonies after drug exposure in vitro — 1-hr *exposure*

		Cisplatin (μg/ml)			CHIP (μg/ml)			CBDCA (μg/ml)			TNO-6 (μg/ml)		
		10	1	0.1	20	2	0.2	50	5	0.5	1	0.1	0.01
Tumour passage	PE%	Colony growth as % of controls											
OvP													
4/5	0.31			43			67			44		6	52
2	0.39	7	50	106	0	42	84	11	70	105	16	34	74
3	0.37	15	45	94	13	72	111	91	112	119	8	30	92
3	0.60	3	50	82	2	60	116	3	23	77	0	10	96
1	0.58	5	82	98	4	43	108	10	44	80	5	34	101
OvL													
7	0.10	7	81	144	11	42	90	36	49	119	18	32	79
8	0.28	1	3	71	0	59	132	0	32	121	5	3	125
Lu1													
6	0.06	48	92	139	72	59	94	54	119	82	6	58	68
7	0.15	3	82	102	0	94	109	18	106	107	0	87	81
Lu2													
5	0.10	154	190		65	165	138	139	112	162	119	146	142
5	0.06	67	75	90	46	72	81	43	54	68	36	80	101
Lu4													
10	0.18			83			87			78		18	5
10	0.06		40	80		50	102		39	93	42	39	
ColH													
3	0.20	45	38	86	0	68	98	0	23	86	12	6	64
4	0.38			59			33			92		22	74
5	0.06					303	175				177	80	17
ColF													
4	0.18	58	58	151	61	58	90	20	47	116	45	47	94
Ys1													
5	0.21	40	39	66	0	78		0	49	86	0	0	66
Adr1													
1	0.05	0	3	52	1	51	65	0	18	50	0	3	39

Tests are listed for each tumour in the order in which they were performed; later tests from early passages were occasionally made from tumours grown in nude mice from material stored in liquid nitrogen. Tumour type coding: Ov = ovary, Lu = lung, Col = colon, Ys = yolk sac, Adr = adrenal. PE% gives the % plating efficiency in the control dish in which 100,000 cells were plated.

Table 3. Growth of human tumour cell colonies after drug exposure in vitro — *continuous exposure*

Tumour passage	PE%	Cisplatin (μg/ml) 1	0.1	0.01	CHIP (μg/ml) 2	0.2	0.02	CBDCA (μg/ml) 5	0.5	0.05	TNO-6 (μg/ml) 1	0.1	0.01
		Colony growth in % of controls											
OvP													
5/6	0.33	0	0		0	39		0	4		0	25	
2	0.39	80	75	102	23	29	43	46	71	105	13	61	77
3	0.59	64	116	105	16	32	68	11	27	80	6	30	71
OvL													
6	0.08	84	108	97	8	19	74	42	62	94	0	21	54
7	0.15	66	51	82	0	19	56	41	41	101	0	0	31
8	0.21	0	23	66	0	27	48		44	83	0		69
Lu1													
6	0.06	10	11	79	0	1	96	99	81	74	36	8	5
7	0.16	55	78	97	20	69	114	47	19	94	14	25	76
Lu2													
5	0.19	81	101	98	79	95	85	50	74	92	44	75	55
5	0.04	31	79	57	326	67	63	67	120	81	44	78	81
Lu3													
8	0.40	43	56	68	0	29	45	8	41	93	0	15	42
Lu4													
10	0.20	2	44		0	37		2	25		0	8	
10	0.06		54	54		78	77		78	93		51	78
ColH													
3	0.21	0	32	53	57	53	64	10	23	54	26	38	54
4	0.51	0	9		0	21		2	6		7	23	
5	0.17				47	41	33				95	105	67
ColF													
4	0.26	58	60	107	36	35	15	13	27	41	46	34	44
3	0.13	0	0	0	0	0	0	0	33	0	13	44	18
Ysl													
5	0.17	0	38	69	52	34	46	0	31	89	0	61	67
Adrl													
1	0.10	0	0		0	4	34		2	0			0
1	0.04	0	0	49	0	1	15	0	0	16	0	0	0

Legends as for Table 2.

point to TNO-6 as a hundredfold more effective agent than the other compounds. In contrast, results after continuous exposure suggest approximately equal effectiveness of the four drugs. When similar tests were performed on mouse leukaemia L1210, which is about equally sensitive to the four drugs, it became clear that cisplatin, CHIP and CBDCA need higher drug concentrations for testing than the assumed human peak plasma concentration (PPC) to cause 75% inhibition of colony formation after a 1-hr exposure (Table 5). For TNO-6 the effective concentration is slightly lower than the PPC. The four drugs are more similar in effectiveness against L1210 cells when tested by continuous exposure, just as against the human tumours.

DISCUSSION

The results lead to a number of simple conclusions on the subrenal capsule assay; in this case the type of drug exposure is adequate and the duration of exposure is not artificially restricted. The problem lies with the inadequacy of the endpoint of the test. Although an occasional test may appear dependable, as the result for tumour Lu4 in Table 1 indicates, this is too rare for predictive testing of individual patient tumour sensitivity. For that purpose the test is not suitable. The unpredictable admixture of non-tumour cells to the volume measured as an endpoint of the test [10] is the most likely explanation for the apparent random variation of test results.

The clonogenic assay has a satisfactory repro-

Table 4. Positive responses in the clonogenic assay dependent on drug concentration

Drug concentration (μg/ml)	Cisplatin	CHIP	CBDCA	TNO-6
	1-hr *exposure*			
50			9/14	
20		10/14		
10	8/14			
5			3/15	
2		0/16		
1	2/15			11/16
0.5			0/18	
0.2		0/18		
0.1	0/17			9/19
0.01				2/18
	Continuous exposure			
5			10/17	
2		14/20		
1	10/19			14/19
0.5			9/20	
0.2		10/21		
0.1	7/20			10/19
0.05			3/17	
0.02		3/18		
0.01	1/16			4/18

Table 5. Sensitivity of mouse leukaemia L1210 to platinum compounds in vitro

Drug	PPC*	Dose causing 75% growth inhibition (μg/ml) 1-hr exposure	Continuous exposure
Cisplatin	2	11.3	0.75
CHIP	4	69.3	2.28
CBDCA	10	226.6	5.80
TNO-6	2	0.60	0.20

*PPC = estimated peak plasma concentration in man in μg/ml.

ducibility of responses. It has, however, two drawbacks: it is applicable only to half of the tumours that grew in nude mice, a population of tumours that already represents a selection from the total number of human malignancies, and furthermore, from our data it is clear that the choice of one-tenth peak plasma concentration as the critical test level for the clinically used platinum derivatives is about one hundredfold too low. This conclusion is not only based on our results with L1210 leukaemia, but it is in agreement with many other recent studies on the effectiveness of cisplatin *in vitro*; see Table 6. This finding also suggests that for other agents a critical review of the selection of suitable test concentrations might be desirable.

In addition, the responses to a small fraction of the tumours indicate a specific type of error. This is most pronounced in the response of colon tumour F in passage 3; not only are the responses clearly different from those of the duplicate test on passage 4; the results are internally inconsistent in indicating an allegedly higher sensitivity to CBDCA and TNO-6 at a lower drug concentration. This type of result, which is also seen after permanent exposure of Lu1 to the same agents, is associated with poor agreement between duplicate tests. This type of anomalous dose–effect relationship seems to indicate poor growth of the tumour cells *in vitro* and is associated with poor agreement of duplicate tests. The result of a single test giving this type of response should be considered unreliable.

This type of response with higher doses appearing less effective has been noted especially in cultures from primary tumours; in model studies with long-passaged cell lines it has not been described. For this reason, it would seem likely to occur even more frequently in primary passages, and it seems likely that this phenomenon was

Table 6. Cisplatin concentrations used in clonogenic assays

Type of tumour	μg/ml Tested	Critical	Reference
Ovarian carcinomas	0.02–0.56	—	[11]
Myeloma	0.01–0.1	0.1	[12]
Primary human tumours	0.05–1.0	0.2	[13]
Primary human tumours	2	2	[14]
Hep-2 cell line	0.02–20	2	[15]
Human cell lines	0.01–2	2	[16]
Xenograft passage	5–15	5	[17]
Tissue culture lines	0.1 –10	10	[5]
Early tumour passages	0.1 –10	10	this study

responsible for the curious observation that those who first reported on clinical responses to cisplatin [11, 13] have used exposure concentrations that were ineffective in experimental studies (see Table 6).

As to the question of whether the response pattern of the three compounds presently being compared in clinical use, cisplatin, CHIP and CBDCA, is different, this study is too small in size to give a clear answer. It cannot exclude a difference, but it can at least be stated that differences in response to these three drugs were not registered more frequently than differences between replicate tests of the same compound. This leaves the possibility of a similarity in response to these three agents open.

REFERENCES

1. Salmon SE. Preclinical and clinical application of chemosensitivity testing with a human tumor cloning assay. In: Salmon SE, Trent JM, eds. *Human Tumor Cloning*. New York, Grune & Stratton, 1984, 499–508.
2. Bogden AE, Cobb WK, LePage DJ *et al.* Chemotherapy responsiveness of human tumors as first transplant generation xenografts in the normal mouse: six-day subrenal capsule assay. *Cancer* 1981, **48**, 10–46.
3. Hamburger AW, Salmon SE. Primary bioassay of human tumor cells. *Science* 1977, **197**, 461–463.
4. Hamburger AW, Salmon SE, Kim MB *et al.* Direct cloning of human ovarian carcinoma cells in agar. *Cancer Res* 1978, **38**, 3438–3444.
5. Wilson AP, Ford CHJ, Newman CE, Howell A. A comparison of three assays used for the *in vitro* chemosensitivity testing of human tumours. *Br J Cancer* 1984, **49**, 57–63.
6. Edelstein MB, Fiebig HH, Smink T, Van Putten LM, Schuchardt C. Comparison between macroscopic and microscopic evaluation of the tumour responsiveness using the subrenal capsule assay. *Eur J Cancer Clin Oncol* 1983, **19**, 995–1009.
7. Courtenay VD. A soft agar colony assay for Lewis lung tumour and B16 melanoma taken directly from the mouse. *Br J Cancer* 1976, **34**, 39–45.
8. Hug V, Spitzer G, Drewinko B, Blumenschein GR. Effect of diethylaminoethyl-dextran on colony formation of human tumor cells in semisolid suspension cultures. *Cancer Res* 1982, **38**, 210–213.
9. Lelieveld P, Van Putten LM. Biologic activity of two derivatives and six possible metabolites of cyclophosphamide (NSC 26271). *Cancer Treat Rep* 1976, **60**, 373–379.
10. Edelstein MB, Smink T, Ruiter DJ, Visser W, Van Putten LM. Improvements and limitations of the subrenal capsule assay for determining tumour sensitivity to cytostatic drugs. *Eur J Cancer Clin Oncol* 1984, **20**, 1549–1556.
11. Hamburger AW, Salmon SE, Kim MB, Trent JM, Soehnlen BJ, Alberts DS, Schmidt HJ. Direct cloning of human ovarian carcinoma cells in agar. *Cancer Res* 1978, **38**, 3438–3444.
12. Durie BGM, Young LA, Salmon SE. Human myeloma *in vitro* colony growth: interrelationship between drug sensitivity, cell kinetics, and patient survival duration. *Blood* 1983, **61**, 929–934.
13. Von Hoff DD, Casper J, Bradley E, Sandbach J, Jones D, Makuch R. Association between human tumor colony-forming assay results and response of an individual patient's tumor to chemotherapy. *Am J Med* 1981, **70**, 1027–1032.
14. Kern DH, Tanigawa N, Bertelsen CA, Sondak VK, Morton DL. Heterogeneity of chemosensitivity response of human tumors. In: Salmon SE, Trent JM, eds. *Human Tumor Cloning*. New York, Grune & Stratton, 1984, 173–191.

15. Clark GM, Von Hoff DD. Quality control of a multicenter human tumor cloning system: the Southwest Oncology Group experience. In: Salmon SE, Trent JM, eds. *Human Tumor Cloning*. New York, Grune & Stratton, 1984, 255–265.
16. Hill BT, Whelan RDH, Hoskin LK, Ward BG, Gibby EM. The value of human tumor continuous cell lines for investigating aspects of the methodologies used for *in vitro* drug sensitivity testing. In: Salmon SE, Trent JM, eds. *Human Tumor Cloning*. New York, Grune & Stratton, 1984, 487–496.
17. Jones AC, Stratford IJ, Wilson PA, Peckham MJ. *In vitro* cytotoxic drug sensitivity testing of human tumour xenografts grown as multicellular tumour spheroids. *Br J Cancer* 1982, **46**, 870–879.