16. Wagner JC. *Fundamentals of Clinical Pharmacokinetics*. Hamilton Illinois, Drug Intelligence Publications, 1976.
17. Ritschel WA. *Handbook of Basic Pharmacokinetics*. Hamilton, Illinois, Drug Intelligence Publications, 1976.
18. Corr R, Berger MR, Betsch B, Brix HP, Floride J, Schmähl D. Modulation of cytosolic sexual steroid receptors in autochthonous methylnitrosourea-induced rat mammary carcinoma following application of 2-chloroethylnitrosocarbamoyl-L-alanine linked to oestradiol or dihydrotestosterone. *Br J Cancer* 1990, **62**, 42–47.

Acknowledgements—This study was carried out within the programme of the EORTC Screening and Pharmacology Group.

Eur J Cancer, Vol. 26, No. 8, pp. 898–901, 1990.
Printed in Great Britain

0277–5379/90 $3.00 + 0.00

Activity of Cytostatic Drugs in Two Heterotransplanted Human Testicular Cancer Cell Lines with Different Sensitivity to Standard Agents

Andreas Harstrick, Hans-Joachim Schmoll, Jochen Casper, Hansjochen Wilke and Hubert Poliwoda

Two established human testicular cancer cell lines were used in a mouse xenograft model to assess the antitumour activity of 15 anticancer agents. Line H 12.1 was highly sensitive to cisplatin, bleomycin and vinblastine, resembling non-pretreated testicular tumours, whereas line H 23.1 showed resistance to cisplatin and vinblastine, comparable to tumours with acquired or intrinsic drug resistance. In line H 12.1 several drugs were highly active, including cyclophosphamide, ifosfamide, nimustine and vincristine; carmustine, vindesine, doxorubicin, epidoxorubicin, pirarubicin, mitoxantrone, carboplatin and iproplatin had only moderate activity. In line H 23.1 only cyclophosphamide, ifosfamide, nimustine, vincristine and bleomycin had antitumour activity. These two cell lines represent a useful model for preclinical evaluation of new agents with presumed activity in testis cancer. *Eur J Cancer*, Vol. 26, No. 8, pp. 898–901, 1990.

INTRODUCTION

THE RATE of complete remissions achieved with cisplatin-based combination chemotherapy in non-seminomatous testicular cancer ranges between 85 and 95%, and most patients can expect to be long-term survivors [1–3]. But patients with advanced disease or patients relapsing after first-line chemotherapy have a poor prognosis [4, 5], and all chemotherapy protocols currently in use have serious side-effects [6, 7].

Clinical investigation of new agents in testicular cancer is hampered by the numbers of eligible patients being too small, which makes preclinical evaluation of new drugs important. Human tumour tissue, transplanted into congenitally athymic nude mice, is a valid and reliable test system for *in vivo* evaluation of chemotherapeutic drugs. The heterotransplanted tumours preserve their histological and biological characteristics. Furthermore, the response of xenografted tumours is correlated with clinical response [8–11].

We have used two established human testicular cancer cell lines with differences in response to standard drugs to assess the activity of a panel of new agents.

Correspondence to A. Harstrick.
The authors are at the Department of Hematology and Oncology, University of Hannover Medical School, Konstanty-Gutschow-Str. 8, 3000 Hannover 61, F.R.G.

MATERIALS AND METHODS

Cell lines

Cell lines H 12.1 and H 23.1 were both established by J.C. in our laboratory. The origin and histology after heterotransplantation of both lines are shown in Table 1. Neither of the two patients had received chemotherapy before orchiectomy. The cell lines were grown as continuous monolayer cultures in RPMI 1640 supplemented with 15% fetal calf serum (Biochrom), penicillin 2 IU/ml, streptomycin 2 μg/ml and L-glutamine

Table 1. Cell line characteristics

	Cell line	
	H 12.1	H 23.1
Origin, date of establishment	Testis, 21/5/81	Testis, 27/4/84
Primary histology	S,EC,T,CC	EC
Histology after heterotransplantation	EC,T,STGC	EC,YS
Doubling-time after heterotransplantation (days)	13.5	13

S = seminoma, T = teratoma, EC = embryonal carcinoma, CC = chorioncarcinoma, STGC = syncytiothrophoblastic giant cells and YS = yolk sac.

Table 2. Maximally tolerated dosages

Drug	Dosage (mg/kg per day)	Schedule (days)
Cisplatin	3	IP (1–5)
Carboplatin	12.5	IP (1–5)
Iproplatin	4.3	IP (1–5)
Cyclophosphamide*	300	IP (1, 15)
Ifosfamide*	600	IP (1, 15)
Nimustine	30	IV (1)
Carmustine	25	IV (1)
Vincristine	1.25	IV (1, 8, 15)
Vinblastine	2.5	IV (1, 8, 15)
Vindesine	1.5	IV (1, 8, 15)
Doxorubicin	3.5	IV (1, 5, 9)
Epidoxirubicin	4.0	IV (1, 5, 9)
Pirarubicin	3.5	IV (1, 5, 9)
Mitoxantrone	2.4	IV (1, 5, 9)
Bleomycin	50	IV (1, 5, 9)

*Mesna was given at the same mg/kg dosage intraperitoneally with cyclophosphamide and ifosfamide.
IP = intraperitoneal, IV = intravenous.

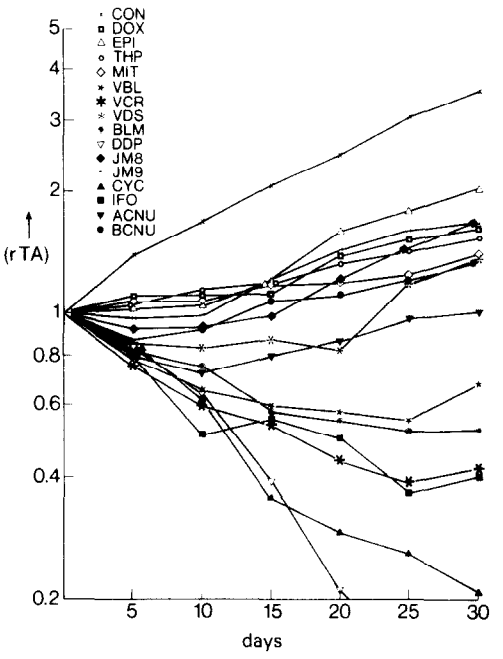

Fig. 1. Antitumour activity of 15 drugs against xenografts of line H 12.1 expressed as relative tumour area vs. days after start of treatment. Each point represents median of 5–8 animals. rTA of cisplatin-treated tumours was 0.13 at day 30. CON = control, DOX = doxorubicin, EPI = epirubicin, THP = pirarubicin, MIT = mitoxantrone, VBL = vinblastine, VCR = vincristine, VDS = vindesine, BLM = bleomycin, DDP = cisplatin, JM8 = carboplatin, JM9 = iproplatin, CYC = cyclophosphamide, IFO = ifosfamide, ACNU = nimustine and BCNU = carmustine.

0.04 mmol/l. There were no significant changes in histology, cytogenetics, growth kinetics or response to chemotherapy over many passages.

Mice

Male athymic (nu/nu) NMRI-mice were used. They were kept in pathogen-free conditions, and fed on an autoclaved standard diet ('Altromin') and given free access to sterilised water.

Drugs

We used commercially available drug preparations: cisplatin, carboplatin, iproplatin and carmustine (Bristol-Myers); cyclophosphamide, ifosfamide and nimustine (ASTA); doxorubicin and 4-epidoxorubicin (Farmitalia); pirarubicin (Behring); mitoxantrone (Lederle); vincristine, vinblastine and vindesine (Lilly); and bleomycin (Mack). All drug solutions were freshly prepared before administration. All drugs were given at equitoxic doses, which were determined in separate experiments in non-tumour bearing mice. The maximally tolerated dose was defined as the dosage that did not kill more than one out of five mice and did not produce more than 20% body weight loss over 30 days (Table 2).

Heterotransplantation and treatment

Cells were harvested by trypsination to obtain single-cell suspensions. The number of viable cells was counted by trypan-blue exclusion and the cells were resuspended in growth medium at 5×10^7 viable cells per ml. 0.2 ml of this suspension was injected subcutaneously into the right flank of the mice. The tumours were measured regularly and the cross-sectional area was calculated (length × width). When most tumours had reached 1–1.5 cm^2 the mice were stratified by tumour size, divided into groups of 5–8 and randomized for treatment.

After the start of treatment, the tumours were measured every 5 days and growth curves were plotted by the relative tumour volume at each given day. 30 days after the start of treatment antitumour activity was assessed by calculating the percentage of tumour volume reduction compared with untreated control mice according to the formula: rVR = 100 − (rVT/rVC × 100). (rVR = relative volume reduction, rVT = relative volume of treated group and rVC = relative volume of controls; volume was calculated by $a \times b^2 \times 0.5$.) A drug with rVR over 75% was considered highly active (++), a drug with rVR 50–75% was active (+) and a drug with rVR below 50% was inactive.

RESULTS

Standard agents

The cell lines differed considerably in their response to the standard drugs for testicular cancer, such as cisplatin, vinblastine and bleomycin (Figs. 1 and 2). H 12.1 was highly sensitive to all three drugs with rVRs of 96%, 81% and 85%, respectively (Table 3). In contrast, H 23.1 showed resistance to cisplatin (rVR 47%) and vinblastine (rVR 51%); bleomycin was still active (rVR 76%). The results obtained with the other agents must therefore be assessed in the context of these differences when H 12.1 might be taken as a model for a drug-sensitive tumour and H 23.1 for a drug-resistant tumour.

Alkylating agents

Cyclophosphamide and ifosfamide had antitumour activity in the cisplatin-sensitive line H 12.1. In this cell line both nitrosoureas were inferior to the oxazaphosphorines. In H 23.1 the activity of cyclophosphamide, ifosfamide and, to a lesser degree, nimustine was lower than that in line H 12.1, indicating a possible cross-resistance between these alkylating agents and cisplatin.

Vinca alkaloids

The three vinca alkaloids differed in their activity in H 12.1. Vincristine was more active than vinblastine or vindesine (rVRs

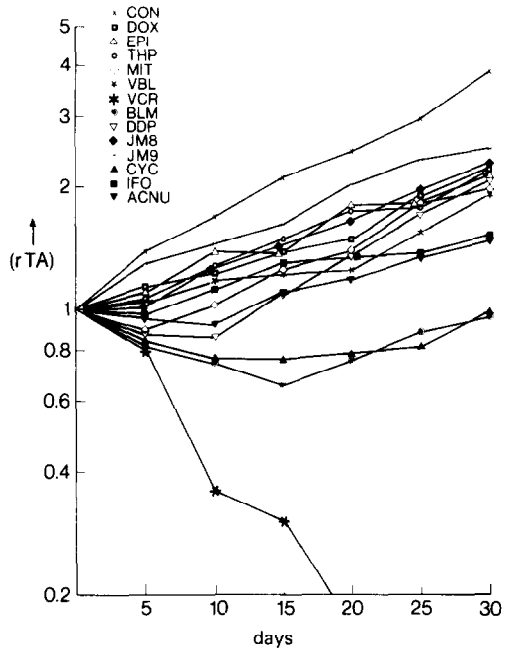

Fig. 2. Antitumour activity of 13 drugs against xenografts of line H 23.1. rTA of vincristine-treated tumours was 0.07 at day 30.

88%, 81% and 60%). Vincristine also showed strong antitumour activity in H 23.1. As in line H 12.1 vinblastine was again inferior to vincristine (rVR 98% vs 51%).

Platinum compounds

Both carboplatin and iproplatin were inferior to cisplatin in the drug-sensitive line H 12.1. In H 23.1 all three platinum analogues were equally ineffective. In two more cisplatin-sensitive testicular cancer cell lines (2102 EP and 1428 A) carboplatin and iproplatin have shown significantly lower antitumour activity than cisplatin [12].

Anthracyclines

When tested in equitoxic concentrations doxorubicin, epidoxorubicin, pirarubicin and mitoxantrone showed almost identical antitumour activity in both lines. In H 12.1 the activity of the anthracyclines was inferior to that of the alkylating agents, cisplatin, bleomycin or the vinca alkaloids. Nevertheless, in the more resistant line H 23.1, the anthracyclines showed antitumour activity that was similar to cisplatin or vinblastine.

Table 3. Antitumour activity in terms of relative volume reduction (rVR)

	H 12.1	H 23.1
Cisplatin	96.1 (++)	47.4 (−)
Carboplatin	52.1 (+)	42.1 (−)
Iproplatin	54.4 (+)	38.4 (−)
Cyclophosphamide	94.3 (++)	75.3 (++)
Ifosfamide	88.6 (++)	61.3 (+)
Nimustine	71.5 (+)	62.8 (+)
Carmustine	60.7 (+)	—
Vincristine	88.0 (++)	98.2 (++)
Vinblastine	80.9 (++)	51.4 (+)
Vindesine	60.1 (+)	—
Doxorubicin	52.7 (+)	46.1 (−)
Epidoxorubicin	42.5 (−)	47.9 (−)
Pirarubicin	55.5 (+)	45.9 (−)
Mitoxantrone	60.1 (+)	51.6 (+)
Bleomycin	85.2 (++)	75.6 (++)

++ = high activity, + = moderate activity and − = no activiy (see text).

DISCUSSION

We used a xenograft model based on two established testicular cancer cell lines. H 12.1 and H 23.1 were chosen because their sensitivity to standard agents indicated that they might resemble two different clinical situations. H 12.1 can be taken as a model for the majority of non-pretreated testicular cancers with high susceptibility to cisplatin, bleomycin and vinblastine [13–15]. Several of the other drugs under study also showed good results, especially the alkylating agents cyclophosphamide and ifosfamide and the vinca alkaloids. These drugs are efficacious in non-pretreated testicular cancer [16, 17]. Our data suggest that neither of the two newer platinum compounds nor the nitrosoureas or anthracyclines are candidates for replacing these standard drugs as first-line therapy in testicular cancer.

The extraordinary high activity of vincristine in both cell lines deserves further discussion. In the patient the dose-limiting toxicity of vincristine is neurotoxicity [18]. Possibly the observation period of 30 days used in that study was too short to assess the real risk of neuropathy produced by such high doses. With an extended observation period we have found that mice treated with these high doses can develop a palsy of the lower extremities suggestive of peripheral neuropathy. Thus the dosage of vincristine used in these experiments might be too high to assess realistically the true efficacy in the clinic. Nevertheless, even when the possibility of overdosing is considered, vincristine seems to have significant activity even in the presence of cisplatin resistance. This high efficacy and the lack of myelosuppression may make vincristine a useful component of combination chemotherapy protocols.

In contrast to line H 12.1 few of the drugs we tested were active in line H 23.1. The only drugs showing sufficient antitumour activity were bleomycin, cyclophosphamide, ifosfamide, vincristine and, surprisingly, nimustine. Again these results concord with the clinical experience that cure by salvage chemotherapy can almost never be achieved in cases of cisplatin resistance [19, 20]. The rather good activity of nimustine in this resistant tumour makes this drug a suitable candidate for phase II studies.

1. Bosl GJ, Gluckman R, Geller NL, Golbey RB, *et al.* VAB-6: an effective chemotherapy regimen for patients with germ cell tumors. *J Clin Oncol* 1986, **4**, 1493–1499.
2. Logothetis CJ, Samuels ML, Selig DE, *et al.* Cyclic chemotherapy with cyclophosphamide, doxorubicin and cisplatin plus vinblastine and bleomycin in advanced germinal tumors. *Am J Med* 1986, **81**, 219–228.
3. Williams SD, Birch R, Einhorn LH, Irwin L, Greco FA, Loehrer PJ. Treatment of disseminated germ cell tumors with cisplatin, bleomycin and either vinblastine or etoposide. *N Engl J Med* 1987, **316**, 1435–1440.
4. Bosl GJ, Geller NL, Vogelzang NJ, *et al.* Alternating cycles of etoposide plus cisplatin and VAB-6 in the treatment of poor risk patients with germ cell tumors. *J Clin Oncol* 1987, **5**, 436–440.
5. Ozols RF, Ihde DC, Linehan WM, Jacob J, Ostchega Y, Young RC. A randomized trial of standard chemotherapy versus high dose chemotherapy regimen in the treatment of poor prognosis nonseminomatous germ cell tumors. *J Clin Oncol* 1988, **6**, 786–792.
6. Roth BJ, Einhorn LH, Greist A. Long-term complications of cisplatin-based chemotherapy for testis cancer. *Sem Oncol* 1988, **15**, 345–350.

7. van Hoff DD, Reichert CM, Cuneo R. Demyelination of peripheral nerves associated with cis-diammine-dichloroplatinum therapy. *Proc AACR* 1979, **20**, 91.
8. Houghton JA, Taylor DM. Maintenance of biological and biochemical characteristics of human colorectal tumors during serial passages in immunedeprived mice. *Br J Cancer* 1978, **37**, 213–223.
9. Steel GG, Courtenay VD, Peckham MJ. The response to chemotherapy of a variety of human tumor xenografts. *Br J Cancer* 1983, **47**, 1–13.
10. Shorthouse AJ, Peckham MJ, Smyth JF, Steel GG. The therapeutic response of bronchial carcinoma xenografts: a direct patient-xenograft comparison. *Br J Cancer* 1980; **41**, 142–145.
11. Fiebig HH, Schuchhardt C, Heuss H, Fiedler L, Löhr GW. Comparison of tumor in nude mice and in the patients. *Behring Inst Clin Res Comm* 1984, **74**, 343–352.
12. Harstrick A, Casper J, Guba R, Wilke H, Poliwoda H, Schmoll H-J. Comparison of the antitumor activity of cisplatin, carboplatin and iproplatin against established human testicular cancer cell lines *in vivo* and *in vitro*. *Cancer* 1989, **63**, 1079–1083.
13. Yagoda A, Mukherje B, Young C, *et al.* Bleomycin—an antitumor antibiotic; clinical experience in 274 patients. *Ann Intern Med* 1972, **77**, 861–870.
14. Rozencweig M, von Hoff D, Slavik M, Muggia FM. Cis-diamminedichloroplatinum—a new anticancer drug. *Ann Intern Med* 1977, **86**, 803–812.
15. Samuels MC, Howe CD. Vinblastine in the management of testicular cancer. *Cancer* 1970, **25**, 1009–1017.
16. Buckner CD. High dose cyclophosphamide for the treatment of metastatic testicular neoplasms. *Cancer Chemother Rep* 1974, **58**, 709–714.
17. Wheeler BM, Loehrer PJ, Williams SD, *et al.* Ifosfamide in refractory male germ cell tumors. *J Clin Oncol* 1986, **4**, 28–34.
18. Weiss HD, Walker MD, Wiernick PH. Neurotoxicity of commonly used antineoplastic agents. *N Engl J Med* 1974, **18**, 127–133.
19. Trump DL, Horvert L. Etoposide and very high dose cisplatin: salvage therapy for patients with advanced germ cell neoplasms. *Cancer Treat Rep* 1985, **69**, 259–261.
20. Williams SD, Einhorn LH. Etoposide salvage therapy for refractory germ cell tumors: an update. *Cancer Treat Rev* 1982, **9** (Suppl A), 67–71.

Eur J Cancer, Vol. 26, No. 8, pp. 901–905, 1990.
Printed in Great Britain

0277–5379/90 $3.00 + 0.00

Correlation of Drug Response in Patients and in the Clonogenic Assay with Solid Human Tumour Xenografts

Catherine C. Scholz, Dietmar P. Berger, Bernd R. Winterhalter, Hartmut Henß and Heinz-Herbert Fiebig

The potential of evaluating the preclinical response of solid tumours was studied in human tumour xenografts in the clonogenic assay. Tumour specimens surgically removed from cancer patients were implanted subcutaneously into thymusaplastic nude mice. Chemosensitivity of the mouse-grown tumours was tested with a modification of the double-layer soft-agar clonogenic assay . Tumour cells were tested against thirteen established cytostatic drugs at two dosages by continuous exposure. 62 retrospective *in vivo/in vitro* correlations were done. The clonogenic assay predicted correctly for clinical response in 16/27 (59%) and for resistance in 32/35 (91%). These correlation rates were similar to reported data for fresh solid human tumour specimens. The results support the clinical relevance of the nude mouse/clonogenic assay model.
Eur J Cancer, Vol. 26, No. 8, pp. 901–905, 1990.

INTRODUCTION

THE POSSIBILITY of individualizing chemotherapy is of special interest to oncologists since patients with identical histological tumour types frequently respond differently to the same chemotherapeutic regimen. Since 1978 research teams have attempted to assess the chemosensitivity of solid human tumours to various cytostatic agents preclinically with the clonogenic assay developed by Hamburger and Salmon [1–3]. Correct predictions for sensitivity are possible in about 69% and for resistance in 92% of diverse solid human tumours [4]. However, these data are difficult to interpret because of lack of standardisation of the experiments and inadequate quality control measures [5]. The key criterion, 'colony', has been defined by some as a minimum number of cells [6–8] but by others as a grouping of cells requiring a minimum diameter ranging from 40 [9] to 100 μm [10]. *In vitro* sensitivity has commonly been defined as inhibition of growth in the drug-treated plates to less than 30% of controls (T/C value under 30%). However, Link *et al.* [11] used a T/C value of 50%, Ajani *et al.* [12] used 35% and Mann *et al.* [8] used 25%. Relevant drug dosages present another significant problem with up to log 2 differences among various laboratories. Comparison of *in vivo/in vitro* correlation results demands standardisation of these key criteria.

We present *in vivo/in vitro* correlations with the clonogenic assay and with quality control measures and criteria for assay standardisation. Solid human tumour xenografts were the tumour source. The use of tumour material following passage

Correspondence to H.-H. Fiebig.
The authors are at the Department of Internal Medicine I, Division of Hematology/Oncology, University of Freiburg, Hugstetter Str. 55, D-7800 Freiburg, F.R.G.