Urol Res (1985) 13:27–34

Urological Research

Comparison of the Cytotoxic Activities of Chemotherapeutic Drugs Using a Human Bladder Cancer Cell Line

P. J. Hepburn[1*], R. T. D. Oliver[2], P. A. Riley[3], B. T. Hill[4], and J. R. W. Masters[1]

[1] Departments of Histopathology and [2] Oncology, St. Paul's Hospital, Institute of Urology, London, UK
[3] Department of Biochemical Pathology, University College Medical School, London, UK
[4] Laboratory of Cellular Chemotherapy, Imperial Cancer Research Fund, Lincoln's Inn Fields, London, UK

Accepted: April 19, 1984

Summary. Many chemotherapeutic drugs have been used to treat patients with advanced bladder cancer, but few of these have been evaluated adequately in phase II clinical trials. Continuous cell lines provide one means for comparing the in vitro cytotoxicities of anticancer agents. In this study, a continuous cell line derived from a transitional cell cancer of the human bladder, which still produces tumours histologically similar to the tumour of origin on xenotransplantation, was used to measure the in vitro cytotoxicities of twelve chemotherapeutic drugs by clonogenic assay. The most cytotoxic agents tested were methotrexate, mitoxantrone, adriamycin, mitomycin C and cisplatin. These in vitro findings are compatible with the activity of these drugs given systemically as single agents in phase II clinical trials in patients with advanced bladder cancer.

Key words: Bladder cancer, Continuous cell line, Chemotherapy.

Introduction

Chemotherapy is used to treat recurrent superficial and metastatic bladder cancer. Drugs such as cisplatin, adriamycin and methotrexate are known to be active [26, 51], but there are many new agents and analogues of existing compounds which could be of value. Thus, there is a need for a screening system to select the most likely agents for clinical trial. Continuous cell lines derived from bladder tumours might be used for this purpose. Such cells provide a rapid, reproducible and inexpensive means for comparing the cytotoxicities of anticancer agents. The aim of this study was to measure the in vitro sensitivities of RT112, a cell line derived from a transitional cell cancer of the human bladder [27, 28], to twelve chemotherapeutic drugs.

* To whom correspondence should be addressed

Material and Methods

Cell Culture

The cell line RT112 was used over a limited passage range, 35–45, and maintained as a monolayer culture in 25 cm^2 flasks (Nunc, Gibco, Paisley, Scotland) in RPMI 1640 medium (Gibco) supplemented with 5% heat-inactivated foetal bovine serum (Flow, Irvine, Scotland) and 2 mM L-glutamine (Gibco) at 36.5 °C in a humidified atmosphere of 5% CO_2 in air. A single batch of serum was used throughout. Mycoplasma was not detected using nutrient agar culture or aceto-orcein stained monolayers [17, 23].

Colony Forming Assays on Plastic

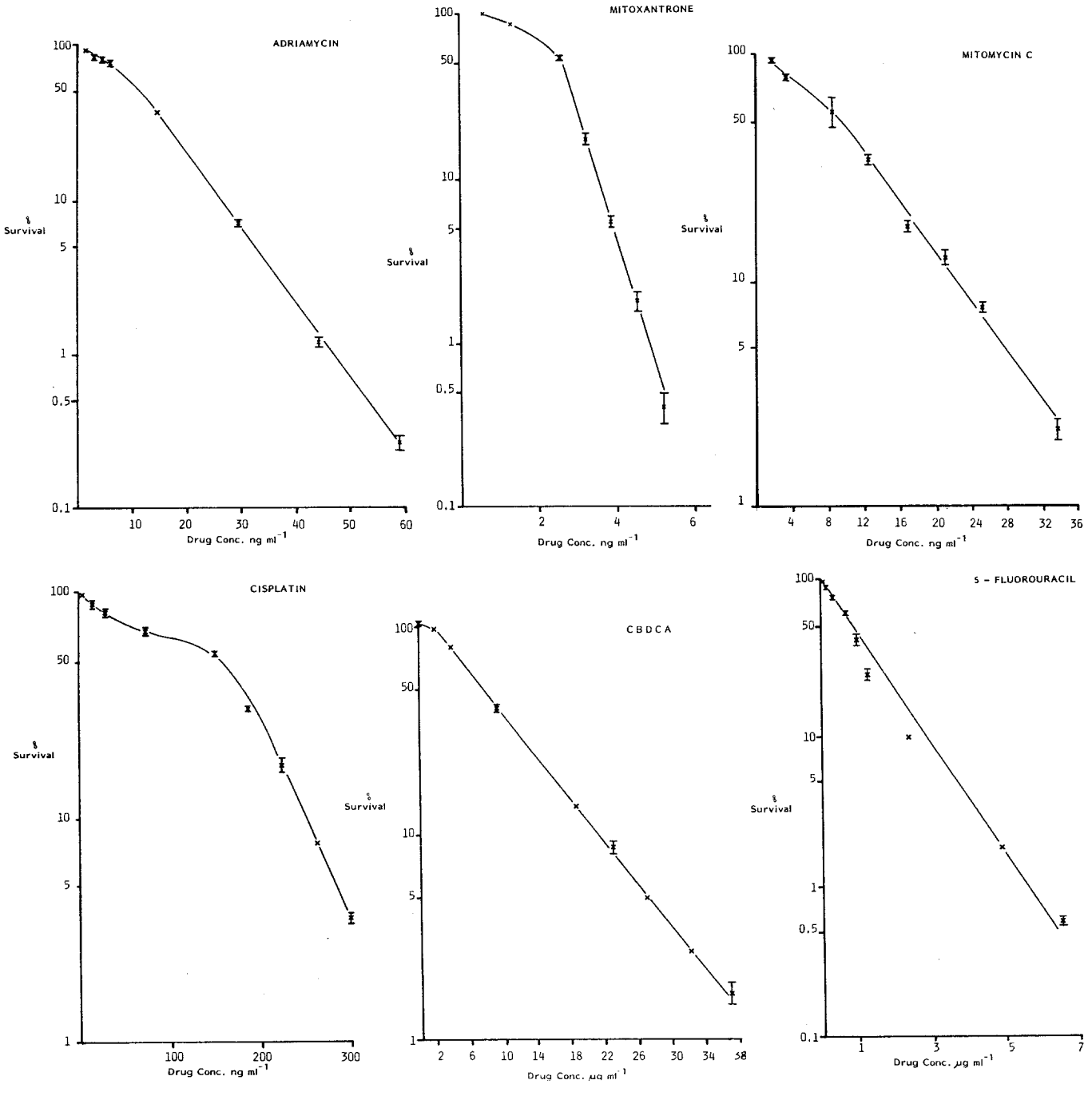
Viable exponentially-growing cells were plated in microtest plate wells (Nunc, 96 wells flat-bottom) at a concentration of 12,800 per well in 0.2 ml medium and incubated at 36.5 °C in a humidified atmosphere of 5% CO_2 in air for 48 h. The medium was then removed and 0.2 ml aliquots of fresh medium containing a range of drug concentrations were added to the exponentially-growing cells (3 replicates per drug concentration). Following a 24 h exposure the cells were washed three times each with 0.2 ml medium and once with calcium and magnesium free phosphate buffered saline (PBSA) [21]. The cells were then detached using 0.2 ml aliquots of 0.01% trypsin (Difco, 1:250, London, England) in 0.003% versene (Ethylenediaminetetra – acetic acid disodium salt, EDTA) (BDH Chemicals, Poole, England), and diluted as necessary in medium to produce cell densities yielding 100 to 200 colonies after plating in 5 cm dishes (Nunc) containing 5 ml medium. This medium was replenished after 7 days and at 14 days the colonies were fixed in methanol and stained with 10% Giemsa (BDH). Colonies of 50 or more cells were scored using a binocular dissecting microscope. The mean colony forming ability of drug-treated cells was expressed as a percentage of the untreated controls. The data are derived from a minimum of three experiments using each drug.

Drugs

Stock solutions of drugs were made up just prior to use in PBSA and diluted in medium to yield a final concentration of PBSA not exceeding 0.1%. The only exceptions were 4-hydroxyanisole (4OHA), which was dissolved directly in medium and dibromodulcitol (DBD), which was solubilised in dimethyl sulphoxide (BDH) and then di-

Table 1. A summary of the drugs used and their sources

Drug	Abbreviation	Source
Adriamycin	ADR	Farmitalia Carlo Erba, Barnet, Herts., UK
Bleomycin	BLM	Lundbeck Ltd., Luton, Beds., UK
Cis-Diammine-1,1-cyclobutane dicarboxylate platinum (II)	CBDCA	Gift from Dr. A. H. Calvert, Institute of Cancer Research, Sutton, Surrey, UK
Cis-Dichlorodiammine platinum (II)	Cisplatin	Mead Johnson Laboratories, Bristol Myers Co., Ltd., Langley, UK
Dibromodulcitol	DBD	Gift from Chinoin Pharmaceutical Works, Budapest, Hungary
5-Fluorouracil	5FU	Roche Products Ltd., Welwyn Garden City, Herts, UK
4-Hydroxyanisole	4OHA	Koch-Light Ltd., Colnbrook, Bucks., UK
Methotrexate	MTX	Lederle Laboratories, Cyanamid, Gosport, UK
Mitomycin C	Mit C	Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan
Mitoxantrone	MX	Gift from Lederle Laboratories, Cyanamid, Gosport, UK
Spirogermanium	SP	Gift from Unimed Inc., Somerville, New Jersey, USA
4-demethyl-epipodophyllotoxin-β-D-ethylidene glucoside	VP16-213	Mead Johnson Laboratories, Bristol Myers Co., Ltd., Langley, UK



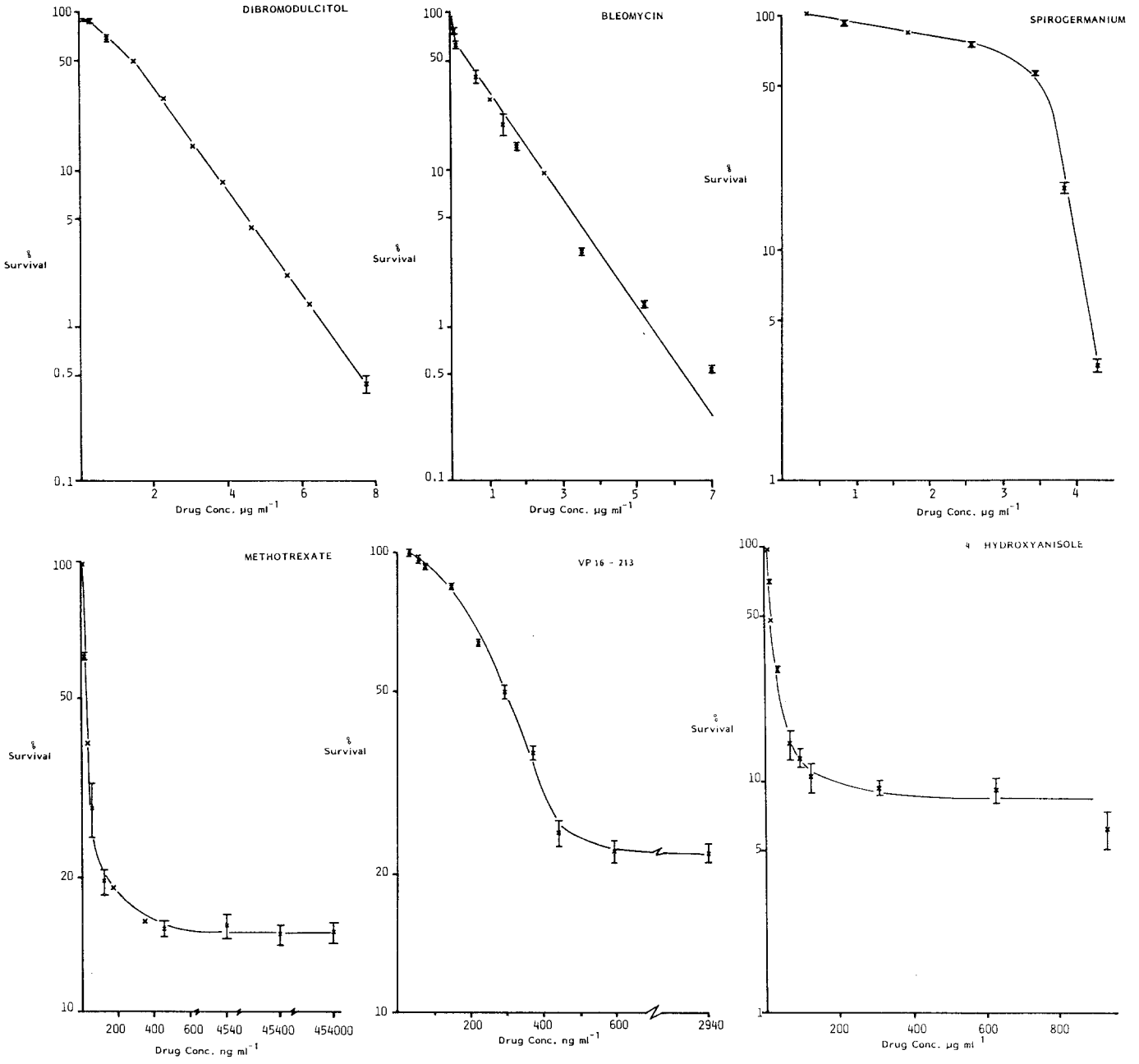


Fig. 1. Each diagram shows clonogenic cell survival of RT112 cells on a logarithmic scale against a range of concentrations of each drug plotted on a linear scale. The concentrations of adriamycin, mitoxantrone, mitomycin C, cisplatin, methotrexate and VP16-213 are plotted in ng/ml, while the remaining drugs are plotted in μg/ml. Each point is the mean of at least three experiments, and only standard error bars in excess of 5% are included

luted at least 1,000-fold in medium before us. The drugs used and their sources are listed in Table 1.

Xenotransplantation

Male nude mice (nu/nu) were injected subcutaneously on one flank with 2×10^7 cells. Tumours approximately 1 cm^3 in size grew within 3–5 weeks and were excised and fixed in Baker's formol calcium. Following routine histological processing paraffin sections were stained with haematoxylin and eosin and examined using light microscopy.

Results

The response of RT112 cells to the twelve anticancer agents is shown in Fig. 1 in which clonogenic cell survival is plotted on a logarithmic scale against drug concentration on a linear scale. Three drugs, methotrexate, 4-hydroxyanisole and VP16-213, produced dose-response curves consisting of an exponential phase followed by a plateau phase. The plateau phase indicates that a fraction of the clonogenic cells were resistant to these drugs during a 24 h exposure,

Table 2. Comparison of in vitro concentrations of each drug required to reduce clonogenic cell survival by 70% (ID70), expressed in ng ml^{-1} and as molar concentrations, with C x T and peak plasma concentrations derived from published clinical studies (using the data in Table 3). NA = not available

Drug	ID70 ng ml^{-1}	ID70 Molar concentration	C x T μg-h ml^{-1}	Peak plasma concentration μg ml^{-1}
Mitoxantrone	2.9	5.7×10^{-9}	0.3	0.4–1.0
Mitomycin C	13.7	4.1×10^{-8}	0.1–1.1	0.4–2.8
Adriamycin	16.3	2.8×10^{-8}	0.5–2.0	0.2
Methotrexate	38.6	8.5×10^{-8}	5.3	2.8–4.5
Cisplatin	196.0	6.5×10^{-7}	5.4	0.3
VP16-213	400.0	6.8×10^{-7}	38–114	4.8–21.6
Bleomycin	1,040.0	7.4×10^{-7}	0.5–5.0	0.1–4.0
5-Fluorouracil	1,475.0	1.1×10^{-5}	16.3	40
Dibromodulcitol	2,150.0	7.0×10^{-6}	5.1–13.4	1.2–17.5
Spirogermanium	3,710.0	1.1×10^{-5}	0.26	0.6
CBDCA	11,800.0	3.2×10^{-5}	146.5	NA
4-Hydroxyanisole	27,000.0	2.2×10^{-4}	38.5	85–97

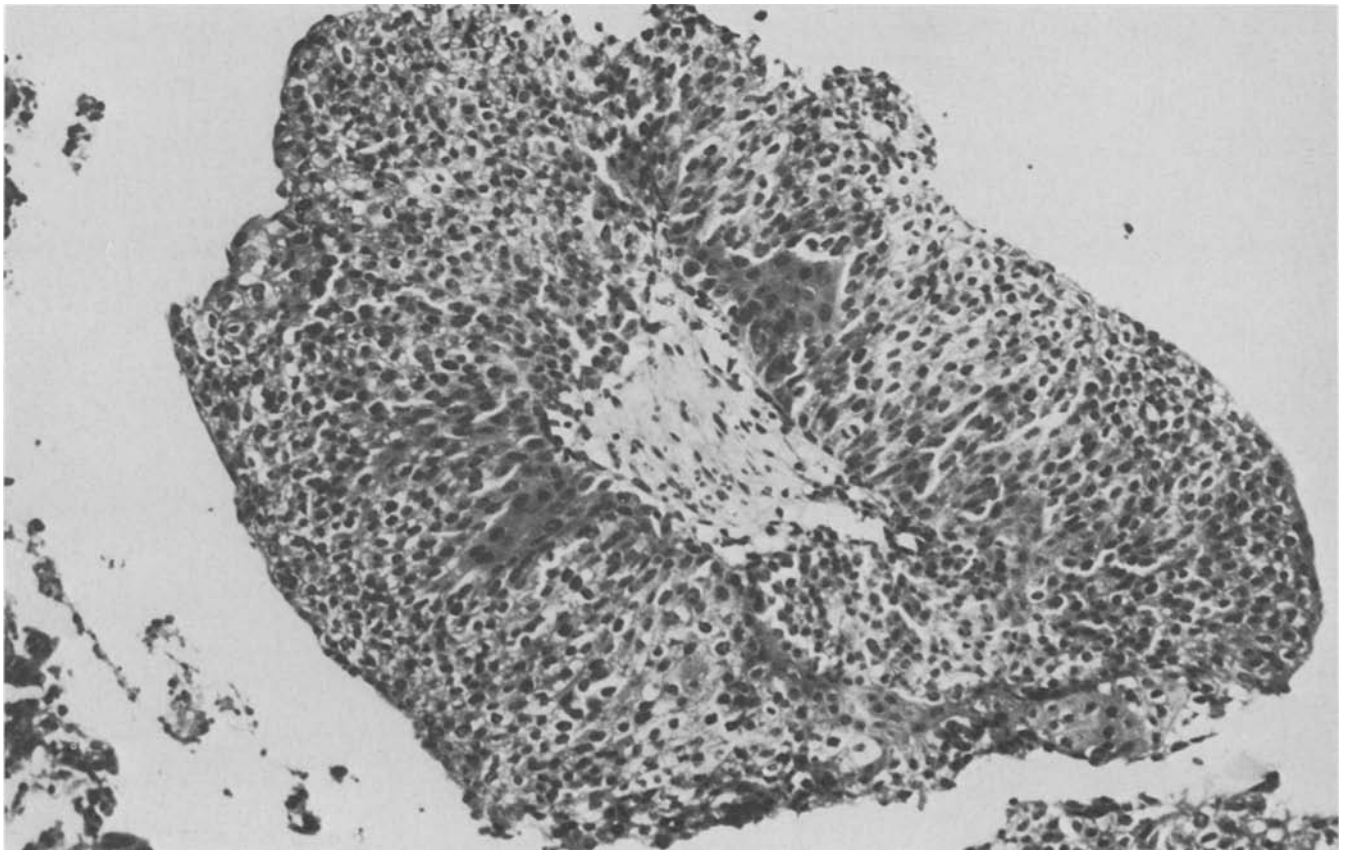


Fig. 2. Histological section of xenograft produced in nude mouse (nu/nu) four weeks after subcutaneous injection of 2×10^7 RT112 cells, H and E, magnification x210

the proportion being approximately 15, 10 and 23% for methotrexate, 4-hydroxyanisole and VP16-213 respectively. In contrast, exponential dose-response curves were seen with 5-fluorouracil and, following a small shoulder region at low drug concentrations, with adriamycin, mitomycin C, dibromodulcitol, CBDCA and mitoxantrone. Spirogermanium and cisplatin also produced exponential dose-response curves, but the shoulder region at low drug concentrations was more pronounced. Bleomycin produced a biphasic exponential dose-response curve.

The doses required to reduce clonogenic cell survival by 70% (ID70) were derived from these dose-response curves,

Table 3. A summary of some of the published data describing the pharmacokinetics in patients of the drugs used in this study, including the doses and routes of administration, measurements of the ranges of peak plasma concentrations, concentration x time characteristics and terminal half-lives. The abbreviations used are: IV, intra-venous; IA, intra-arterial; IM, intra-muscular; NA, not available

Drug	Dose	Route of Administration	Peak Plasma Concentrations $\mu g\ ml^{-1}$	C x T Range $\mu g\ h\ ml^{-1}$	Terminal Half-Life h	Reference
Adriamycin	60 mg/M^2 2–10 min infusion	IV	0.22	1.51	28.1	[10]
	75 mg/M^2 15 min infusion	IV		0.54–1.95	30.1	[24]
Bleomycin	2–10 mg/M^2	IM	0.13–0.59	0.5–3.4	2.3–3.0	[41]
	15 mg/M^2	IV	2–4	5.0	4.0	[2]
CBDCA	400 mg/M^2 1 h infusion	IV	NA	146.5	5.9	[13]
	20–320 mg/M^2 24 h infusion	IV			2.8	[16]
Cisplatin	100 mg/M^2 24 h infusion	IV	0.3	5.4	0.4	[50]
Dibromodulcitol	15 mg/kg	Oral	17.5		8	[8]
	15 mg/kg	Oral	1.2	5.1–13.4	8	[36]
5FU	460–525 mg/M^2 Bolus	IV			0.7	[38]
	15 mg/kg	IV	40.0	16.3	0.2	[22]
4OHA	57 mg/kg Bolus	IA	85.0–97.0	38.5	0.28	[40]
Methotrexate	30 mg/M^2	IV	2.75	5.3	7.6	[11], [3]
	100 mg/M^2		4.5		10	[14]
Mitomycin C	10–30 mg/M^2	IV	0.5–2.7	0.1–1.1	0.15–0.3	[15], [3]
	10–20 mg/M^2	IV	0.4–2.8	0.2–1.1	0.9	[44]
Mitoxantrone	12 mg/M^2 30 min infusion	IV	0.4–1.0		24	[45]
	12 mg/M^2	IV		0.3	2	[5], [6]
Spirogermanium	120 mg/M^2	IV	0.60	Approx. 0.26	NA	[39]
VP16-213	100 mg/M^2	IV bolus		38.2–113.9	4.4–9.6	[18]
	121–214 mg/M^2 oral		4.8–21.6			[18]
		IV (0,5–1 h) infusion			5.7	[1]

and are listed on a weight and molarity basis in Table 2. The molar concentration indicates the number of molecules required to achieve this level of cell kill, and thus drugs with a high molecular weight will appear to be relatively more effective than in comparisons made on a gram weight basis. In addition in Table 2 the plasma concentration time product (C x T) and peak plasma concentrations derived from published pharmacokinetic studies in patients (see Table 3) are listed for each drug.

Following xenotransplantation to nude mice, RT112 cells produced tumours characteristic of transitional cell cancer of the human bladder (Fig. 2).

Discussion

Using a continuous cell line derived from a transitional cell cancer of the human bladder it is shown that there is a greater

than 1,000-fold range in the gram weights of different drugs required to achieve the same level of clonogenic cell kill. These data are highly reproducible and provide a rapid and inexpensive method for comparing the in vitro cytotoxicities of chemotherapeutic drugs. The line RT112 was chosen for this study because it produces characteristic transitional cell cancer following xenotransplantation.

The shapes of the dose-response curves are similar to those described previously for other human cell lines [20, 32]. In addition, the patterns conform approximately to the classification of chemotherapeutic drugs described by Bruce et al. [12]. Cell cycle phase-specific or Class II agents kill cells during a specific phase(s) of the cycle, and therefore cells which do not pass through this phase(s) during the exposure period are spared. This results in an exponential/plateau dose-response curve, as exemplified in this study by methotrexate, 4-hydroxyanisole and VP16-213. Cell cycle specific or Class III agents kill cells throughout the cycle although most are more cytotoxic to proliferating than resting cells [30], thereby producing an exponential dose-response curve as observed with the remainder of the drugs used here. The clinical significance of these cell kinetic differences in the effects of drugs has been reviewed [29]. A slightly different classification of chemotherapeutic drugs into five groups according to the shape of their in vitro dose-response curves has been described by Drewinko et al. [19]. One of these groups is termed threshold exponential, and is characterised by an initial shoulder region at low drug concentrations, which we observed with a number of the agents. This shoulder region could be explained by an accumulation of sublethal damage [19].

Some work has been published previously on the sensitivities of continuous cell lines derived from human bladder cancers to chemotherapeutic drugs. Using the cell line T24 Kato et al. [37] measured uptake of ^{14}C-leucine while Shrivastav and Paulson [48] using the cell line 3176, measured the uptake of radioactive precursors of DNA, RNA and protein. However, it is now generally accepted that clonogenic assay is the only reliable method for measuring the reproductive capacity of cells following exposure to cytotoxic drugs [19, 31, 46, 47]. Clonogenic assays were used by Hagen et al. [25] to measure the in vitro sensitivities of the bladder cell lines MGH-U1, MGH-U2 and RT4, and by Hisazumi et al. [35] for the lines KK47, KW103 and RT4. However, the data for RT4 differs substantially between these two groups, perhaps reflecting differences in culture conditions or the clonogenic assay procedure.

The plasma concentration time product is probably the most important pharmacokinetic parameter in determining drug efficacy and toxicity clinically [4]. In order to compare the in vitro cytotoxicities of the agents used with drug levels measured in patients, the C x T and peak plasma concentrations derived from published studies are listed alongside the ID70s measured in vitro in Table 2. Although there is considerable variation in the published reports, as shown in Table 3, measurements of C x T may give a more accurate representation of drug bioavailability to tumour cells in patients than peak plasma concentrations, because the period during which measurable drug levels are circulating is taken into account. The five most effective drugs in vitro against RT112 cells are mitoxantrone, mitomycin C, adriamycin, methotrexate and cisplatin. Mitoxantrone is an anthracenedione with structural similarities to adriamycin, but theoretically with less cardiotoxic potential [49]. Experimental and early preclinical studies indicate that mitoxantrone has at least comparable antitumour activity to adriamycin [49], and our findings are in accord with this conclusion. CBDCA is a structural analogue of cisplatin, but with less in vitro cytotoxicity on a gram-weight basis. However, much higher concentrations of CBDCA are achievable in patients, and therefore the relative activities are comparable. As CBDCA has significantly less normal tissue toxicity than cisplatin [13], it may therefore also be of value in the treatment of bladder cancer. The cytotoxic activity of spirogermanium against RT112 cells has been described previously [33] and combinations of this agent with 5-fluorouracil or cisplatin were synergistic [34].

Systemic chemotherapy may be used to treat superficial, as well as advanced bladder cancer. In this former case different pharmacokinetic principles apply, and the most effective drugs are likely to be those excreted predominantly in the urine or metabolised to active forms by urothelium. For instance, 90% of an intravenously administered dose of methotrexate is excreted unchanged in the urine within 24 h [7]. Cisplatin also is excreted primarily in the urine, although the fraction of dose eliminated in this manner appears to depend on the length of the infusion. Following a 6 h infusion 75% of a total dose of cisplatin was excreted in the urine, compared with only 40% following a 15 min infusion [9, 43]. In contrast to cisplatin and methotrexate, less than 15% of adriamycin is excreted in the urine [7]. Of particular interest is 4-hydroxyanisole, which appears to be excreted in the urine as a glucuronide, and the possibility exists that the beta-glucuronidase activity in urothelium could liberate significant amounts of the active drug [40].

For a variety of reasons the value of chemotherapeutic drugs has been difficult to evaluate in patients with advanced bladder cancer, as discussed by Yagoda [51]. Consequently there is little reliable information from phase II clinical trials with large series of patients. Proven activity for single agents in metastatic disease was ascribed only to cisplatin, adriamycin and methotrexate [26, 42, 51], three of the most effective agents in vitro. The other two agents with comparable in vitro cytotoxicities, mitoxantrone, and mitomycin C, have as yet had limited use in advanced bladder cancer.

It is impossible to test every new anticancer agent in combination which becomes available in clinical trials of bladder cancer with statistically meaningful numbers of patients. In addition, the standard experimental screening systems have many limitations, and do not usually include bladder tumours. The current possibilities for selecting agents to test in phase II clinical trials for this disease using human cells are human bladder tumour xenografts, stem cell assays

and continuous cell lines. There are advantages and limitations to each of these models, although continuous cell lines provide the most reproducible, flexible and inexpensive system. In conclusion, we would agree with Hisazumi et al. [35] that an appropriate selection from the available human bladder cancer continuous cell lines might be used as a model system for assessing drugs for the therapy of this disease.

Acknowledgements. We thank Mr. K. A. Wallace and Miss D. E. Bennett for carrying out the xenografting procedure with the nude mice in the Animal House Unit at the Imperial Cancer Research Fund Laboratories, and Miss Virginia Wallis for typing the manuscript.

References

1. Achterrath W, Niederle N, Raettig R, Hilgard P (1982) Etoposide – chemistry, preclinical and clinical pharmacology. Cancer Treat Rev 9:Suppl A, 3–13
2. Alberts DS, Chen HSG, Liu R, Himmelstein KJ, Mayersohn M, Perrier D, Gross J, Moon T, Broughton A, Salmon SE (1978) Bleomycin pharmacokinetics in man. I. Intravenous administration. Cancer Chemother Pharmacol 1:177–181
3. Alberts DS, Chen HSG (1980) Tabular summary of pharmacokinetic parameters relevant to in vitro drug assay. In: Salmon SE (ed) Cloning of human tumor stem cells. Alan R Liss Inc, New York, pp 351–359
4. Alberts DS, Chen HSG, Salmon SE (1980) In vitro drug assay – pharmacologic considerations. In: Salmon SE (ed) Cloning of human tumor stem cells. Alan R Liss Inc, New York, pp 197–207
5. Alberts DS, Peng YM, Davis TP (1981) Comparative pharmacology of mitoxantrone and bisantrene. Proc NCI EORTC Symp New Drug Cancer Ther: Abstract 35
6. Alberts DS, Peng YM, Leigh S, Davis TP, Woodward DL (1983) Pharmacokinetics of mitoxantrone in patients. Proc 13th Int Congr Chemother, Vienna (in press)
7. Balis FM, Holcenberg JS, Bleyer WA (1983) Clinical pharmacokinetics of commonly used anticancer drugs. Clin Pharmacokinet 8:202–232
8. Belej MA, Troetel WM, Weiss AJ, Stambaugh JE, Manthei RW (1972) The absorption and metabolism of dibromodulcitol in patients with advanced cancer. Clin Pharmacol Ther 13:563–572
9. Belt RJ, Himmelstein KJ, Patton TF, Bannister SJ, Sternson LA, Repta AJ (1979) Pharmacokinetics of non-protein-bound platinum species following administration of cis-dichlorodiammineplatinum (II). Cancer Treat Rep 63:1515–1521
10. Benjamin RS, Riggs CE, Bachur NR (1977) Plasma pharmacokinetics of adriamycin and its metabolites in humans with normal hepatic and renal function. Cancer Res 37:1416–1420
11. Bischoff KB, Dedrick RL, Zaharko DS, Longstreth JA (1971) Methotrexate pharmacokinetics. J Pharm Sci 60:1128–1133
12. Bruce WR, Meeker BE, Valeriote FA (1966) Comparison of the sensitivity of normal hematopoietic and transplanted lymphoma colony-forming cells to chemotherapeutic agents administered in vivo. J Natl Cancer Inst 37:233–245
13. Calvert AH, Harland SJ, Newell DR, Siddik ZH, Jones AC, McElwain TJ, Raju S, Wiltshaw E, Smith IE, Baker JM, Peckham MJ, Harrap KR (1982) Early clinical studies with cis-diammine-1,1-cyclobutane dicarboxylate platinum II. Cancer Chemother Pharmacol 9:140–147
14. Chabner BA, Donehower RC, Schilsky RL (1981) Clinical pharmacology of methotrexate. Cancer Treat Rep 65, Suppl 1:51–54
15. Crooke ST, Bradner WT (1976) Mitomycin C: a review. Cancer Treat Rev 3:121–139
16. Curt GA, Grygiel JJ, Corden BJ, Ozols RF, Weiss RB, Tell DT, Meyers CE, Collins JM (1983) A phase I and pharmacokinetic study of diamminecyclobutane-dicarboxylatoplatinum (NSC 241240). Cancer Res 43:4470–4473
17. Dickson C, Elkington J, Hales A, Weiss R (1980) Antibody – mediated neutralisation of virus Is abrogated by mycoplasma. Infect Immun 28:649–653
18. D'Incalci M, Farina P, Sessa C, Molina P, Mangioni C, Jancovich M, Masera G, Beer M, Cavalli F (1982) Pharmacokinetic of VP16 in humans. Chemioterapia I:126–129
19. Drewinko B, Roper PR, Barlogie B (1979) Patterns of cell survival following treatment with antitumor agents in vitro. Eur J Cancer 15:93–99
20. Drewinko B, Patchen M, Yang LY, Barlogie B (1981) Differential killing efficacy of twenty antitumor drugs on proliferating and nonproliferating human tumor cells. Cancer Res 41:2328–2333
21. Dulbecco R, Vogt M (1954) Plaque formation and isolation of pure lines with poliomyelitis viruses. J Exp Med 99:167–182
22. Finn C, Sadée W (1975) Determination of 5-fluorouracil (NSC-19893) plasma levels in rats and man by isotope dilution – mass fragmentography. Cancer Chemother Rep 59:279–286
23. Fogh J, Fogh H (1964) A method for direct demonstration of pleuropneumonia-like organisms in cultured cells. Proc Soc Exp Biol Med 117:899–901
24. Greene RF, Collins JM, Jenkins JF, Speyer JL, Myers CE (1983) Plasma pharmacokinetics of adriamycin and adriamycinol: Implications for the design of in vitro experiments and treatment protocols. Cancer Res 43:3417–3421
25. Hagen K, Daly JJ, Kamali HM, Lin JC, Yu SC, Prout GR (1979) New assay for cytotoxic agents in human bladder cancer. Surg Forum 30:560–562
26. Harker WG, Torti FM (1983) The chemotherapy of bladder carcinoma: Systemic therapy. Recent Results Cancer Res 85:37–49
27. Hastings RJ, Franks LM (1981) Chromosome pattern, growth in agar and tumorigenicity in nude mice of four human bladder carcinoma cell lines. Int J Cancer 27:15–21
28. Hepburn PJ, Masters JRW (1983) The biological characteristics of continuous cell lines derived from human bladder. In: Bryan GT, Cohen SM (eds) The pathology of bladder cancer, vol II. CRC Press Inc, Florida, pp 213–227
29. Hill BT, Baserga R (1975) The cell cycle and its significance for cancer treatment. Cancer Treat Rev 2:159–175
30. Hill BT (1978) Cancer chemotherapy. The relevance of certain concepts of cell cycle kinetics. Biochim Biophys Acta 516: 389–417
31. Hill BT, Whelan RDH, Rupniak HT, Dennis LY, Rosholt MS (1981) A comparative assessment of the in vitro effects of drugs on cells by means of colony assays or flow microfluorimetry. Cancer Chemother Pharmacol 7:21–26
32. Hill BT, Whelan RDH (1981) Assessments of the sensitivities of cultured human neuroblastoma cells to anti-tumour drugs. Pediatr Res 15:1117–1122
33. Hill B, Metcalfe S, Hepburn PJ, Masters JRW, Whelan RDH (1983) Evaluation of spirogermanium in murine and human tumour lines in vitro and demonstration of synergy with cisplatin or 5-fluorouracil. Proc 13th Int Congr Chemother, Vienna: Pt 257, 30–33
34. Hill BT, Bellamy AS, Metcalfe S, Hepburn PJ, Masters JRW, Whelan RDH (1984) Identification of synergistic combinations of spirogermanium with 5-fluorouracil or cisplatin using a range of human tumour cell lines in vitro. Invest New Drugs 2:29–33

35. Hisazumi H, Nakajima K, Uchibayashi T (1983) Chemosensitivity of established human bladder cell lines in vitro. Gann 74: 176–182
36. Horváth IP, Csetényi J, Hindy I, Kerpel-Fronius S, Institoris L, Hegedüs L, Eckhardt S (1982) Metabolism and pharmacokinetics of dibromodulcitol (DBD, NSC-104800) in man – II. Pharmacokinetics of DBD. Eur J Cancer Clin Oncol 18:1211–1219
37. Kato T, Nemato R, Nishimoto T, Kumagai I, Miura K (1979) Chemosensitivity of human bladder cancer cells in long-term culture and clinical responses to the selected anticancer drug. Cancer 44:58–63
38. Kirkwood JM, Ensminger W, Rosowsky A, Papathanasopoulos N, Frei E (1980) Comparison of pharmacokinetics of 5-fluorouracil and 5-fluorouracil with concurrent thymidine infusions in a phase I trial. Cancer Res 40:107–113
39. Mattsson W, Tropé C, Orbert B (1980) A phase I study of Spirogermanium. Proc Am Assoc Cancer Res 21:194
40. Morgan BDG, Holden J, Dewey DL, Riley PA (Unpublished results)
41. Oken MM, Crooke ST, Elson MK, Strong JE, Shafer RB (1981) Pharmacokinetics of bleomycin after 1M administration in man. Cancer Treat Rep 65:485–489
42. Oliver RTD, England HR, Risden RA, Blandy JP (1984) Methotrexate in the treatment of metastatic and recurrent primary transitional cell carcinoma. J Urol 131:483–485
43. Patton TF, Himmelstein KJ, Belt R, Bannisters SJ, Sternson LA, Repta AJ (1978) Plasma levels and urinary excretion of filterable platinum species following bolus injection and IV infusion of cis-dichlorodiammineplatinum (II) in man. Cancer Treat Rep 62:1359–1362
44. Pinedo HM, Bocken MCYM, Gall HE, Den Hartigh J, Van Oort WJ (1982) Analysis and pharmacokinetics of mitomycin C. In: Ogawa M, Rozencweig M, Stavquet MJ (eds) Mitomycin C: Current impact on cancer chemotherapy. Excerpta Medica, Amsterdam Princeton Geneva Tokyo, pp 25–29
45. Reynolds DL, Ulrich KK, Patton TF, Repta AJ, Sternson LA, Myron MC, Taylor SA (1981) Plasma levels of 1,4-dihydroxy-5,8-bis({2-[(2-hydroxylethyl)aminol]ethyl}amino)-9,10-anthracenedione dihydrochloride (DHAD) in humans. Int J Pharm 9:67–71
46. Roper PR, Drewinko B (1976) Comparison of in vitro methods to determine drug-induced cell lethality. Cancer Res 36:2182–2188
47. Rupniak HT, Dennis LY, Hill BT (1983) An intercomparison of in vitro assays for assessing cytotoxicity after a 24 hour exposure to anti-cancer drugs. Tumori 69:37–42
48. Shrivastav S, Paulson DF (1980) In vitro chemotherapy testing of transitional cell carcinoma. Invest Urol 17:395–400
49. Smith IE (1983) Mitoxantrone (novantrone): a review of experimental and early clinical studies. Cancer Treat Rev 10: 103–115
50. Vermorken JB, Van Der Vijgh WJF, Klein I, Gall HE, Pinedo HM (1982) Pharmacokinetics of free platinum species following rapid, 3 hr and 24 hr infusions of cis-diamminedichloroplatinum (II) and its therapeutic implications. Eur J Cancer Clin Oncol 18:1069–1074
51. Yagoda A (1980) Chemotherapy of metastatic bladder cancer. Cancer 45:1879–1888

P. J. Hepburn
Department of Histopathology
St. Paul's Hospital
Institute of Urology
24 Endell Street
London WC2
UK