Cancer Chemother Pharmacol (1988) 21: 31–34

Cancer Chemotherapy and Pharmacology

Application of a new preclinical drug screening system for cancer of the large bowel*

Werner Scheithauer[1], Mary P. Moyer[3], Gary M. Clark[2], and Daniel D. Von Hoff[2]

[1] Department of Gastroenterology and Hepatology II, Vienna University School of Medicine, Vienna, Austria
Departments of [2] Medicine and [3] Surgery, University of Texas Health Science Center, San Antonio, TX, USA

Summary. We report a prospective evaluation of three human, continuous colorectal cancer cell lines and a new semiautomated radiometric technique (Bactec system) as a primary screening procedure for cytotoxic compounds with activity against cancer of the large bowel. COLO 320DM, Ht-29, and the metastatic OM-1 colon cancer cell line that have previously been shown to yield clinically relevant information in terms of drug sensitivity patterns in humans were all tested against 11 new compounds currently being investigated in phase I or early phase II clinical trials. Our results suggest that trimetrexate, DUP-785, didemnin B, and flavone-8-acetic acid may be clinically effective for the treatment of colorectal cancer.

Introduction

We have recently described a new in vitro screening system for determination of the activity of anticancer compounds in colorectal cancer [18]. Basically, this system consists of three human continuous colorectal cancer cell lines (COLO 320DM, OM-1, Ht-29) that have been shown to manifest in vitro responsiveness consistent with that known from clinical trials with a variety of standard anticancer agents. Cytotoxic drug effects are measured by use of a new semiautomated radiometric technique (Bactec system [9]) and are quantitated with attention to clinically achievable peak plasma concentrations.

The aim of the present study was a prospective evaluation of the potential of this screening system by testing a series of new compounds that are currently being investigated in phase I or early phase II clinical trials. In order to overcome the potential limitation of this system in terms of in vitro testing of agents without the availability of accurate human pharmacokinetic information [18], a multiple linear regression model based on the relationship between acute animal toxicity data in mice (LD 50 values) and peak plasma concentrations in humans was used [19]. Our results suggest that trimetrexate, DUP-785, didemnin B, and flavone-8-acetic acid may be clinically effective for treatment of cancer of the large bowel.

Materials and methods

Cell lines. COLO 320DM and Ht-29 cells were obtained from the American Type Culture Collection, Rockeville, Maryland. The human metastatic colonic carcinoma cell line, OM-1, was kindly provided by Dr. D. Dexter, Biochemical Department, E. I. Du Pont de Nemours and Co., Glenolden, Pensylvania. General morphologic and biological properties of the neoplastic cells have been described previously in detail [4, 16, 21]. Cells were cultured in 75-cm^2 plastic flasks (Corning Glass works, Corning, NY) in the appropriate culture medium, supplemented with 10% fetal calf serum (FCS) and gentamicin (5 μg/ml); all tissue reagents were obtained from Grand Island Biological Co. (Grand Island, NY). The two adherent cell lines (OM-1, Ht-29) were passaged by a short exposure to 0.25% trypsin. COLO 320DM cells were subcultured by shaking the flask and pouring a portion of the resultant cell suspension into a new flask containing fresh medium. All drug-exposure studies were performed when the cells were in the late-log or early-stationary phase growth.

Drugs. Didemnin B, flavone-8-acetic acid, and nafidimide were provided by the Drug Developmental Therapeutics Program, National Cancer Institute (Bethesda, Md.). The following compounds were donated for this study: DUP-785 (E. I. Du Pont de Nemours and Co. Wilmington, Del.), fostriecin (CI-920), and trimetrexate by Warner-Lambert (Ann Arbor, Mich.), menogarol by Upjohn, (Kalamazoo, Mich.), and vinzolidine by Lilly Research Laboratories (Indianapolis, Ind.). Alpha-2 recombinant interferon (IF; specific activity 1.8×10^8 U/mg) was obtained by Schering Corp. (Bloomfield, NJ), beta-rec. IF (1.8×10^8 U/mg) by Cetus Corp. (Emeryville, Calif.), and gamma rec. IF (1.4×10^7 U/mg) by Biogen Research Corp. (Cambridge, Mass.). Preliminary information in terms of pharmacokinetically achievable peak plasma concentrations (PPC) in man were only available for six compounds. These data obtained from the published literature [1, 7, 8, 12, 13, 14] are shown in Table 1.

All drugs were solubilized in sterile phosphate-buffered saline; appropriate dilutions (each drug was tested at 10.0, 1.0, 0.1, and 0.01 μg/ml) were prepared immediately before use to prevent loss of activity.

* This study was supported in part by a grant JO042 from the Fonds zur Förderung der Wissenschaftlichen Forschung, Vienna, Austria, a grant from Johnston Laboratories, Towson, Maryland, and by contract NO1-CM-57737 from the Department of Health and Human Services

Offprint requests to: Werner Scheithauer, Department of Gastroenterology and Hepatology II, Vienna University School of Medicine, A-1090 Vienna, Garnisongasse 13, Austria

Table 1. In vitro activity of the compounds with known clinically achievable peak plasma concentrations in man that were screened in this series

Drug	Clinical dosage	Peak plasma concentration (μg/ml)	ID 90 to 1/10 PPC[a]		
			COLO 320DM	OM-1	Ht-29
Alpha-2 rec. IF	0.017 mg i.m.	$0.3-1.8\times10^{-3}$	>1000.00	>1000.00	>1000.00
Beta rec. IF	0.056 mg i.v.	0.2×10^{-3}	>1000.00	>1000.00	>1000.00
Gamma rec. IF	0.286 mg/h i.v.	$2.9-4.3\times10^{-3}$	>1000.00	>1000.00	>1000.00
Menogarol	126 mg/m^2 per h i.v.	0.16	649.36	85.26	554.49
Trimetrexate	140 mg/m^2 i.v.	41.00	1.48	2.13	4.74
Vinzolidine	26–75 mg/m^2 p.o.	0.06–0.27	395.19	446.69	36.67
(5-Fluorouracil	15 mg/kg i.v.	60.00	17.36	6.81	3.81)

[a] In vitro response was defined as an "ID 90 to one-tenth PPC ratio" of less than 30

Chemosensitivity testing. Following trypsinization, tumor cell suspensions were transferred to tubes and adjusted to a final concentration of $3-4\times10^4$ cells/ml in the presence of the appropriate drug dilution or control medium. After incubation for 1 h at 37° C, cells were washed twice in Hank's balanced salt solution plus 10% FCS, and prepared for culture. All drug tests were performed in at least duplicate experiments. For quality control purposes a positive control consisting of chromomycin A3 (100 μg/ml) was used [20]. In order for an experiment to be considered evaluable, the chromomycin had to produce a <30% survival of control growth values. To compare the antitumor effects of the investigational compounds and in an attempt to monitor the chemotherapeutic behavior of the cell lines, 5-FU was routinely included in each series of drug tests. (In vitro sensitivity to 5-FU was previously found for all three cell lines [18]).

Culture technique. The Bactec instrument (Johnston Laboratories, Towson, Md.) was originally developed for the early detection of bacterial growth from patient blood samples. Its applicability has been modified by Von Hoff [10] and Kurnick et al. [11] for measuring tumor cell growth. The basic principle of this metabolic assay system is the measurement of 14 CO_2 produced by tumor cells from 14C-glucose incorporated into the culture medium. A 1.8-ml (cytostatically pretreated or control) cell suspension containing $3-4\times10^4$ cells, plus 0.2 ml of an aqueous solution of 14C-glucose (1 μC/ml) are mixed and injected aseptically into 20-ml glass vials. For an initial adjustment of the gaseous atmosphere in the sealed glass vials (kindly provided by Johnston Laboratories) to 5% CO_2/air, as well as for subsequent measurements of the amount of 14C-glucose metabolism (usually day 3, 6, and 9), the vials are simply placed in the Bactec apparatus. The atmosphere (14 CO_2 containing) is automatically flushed into an ionization chamber and replaced. The amount of 14 CO_2 measured is directly converted into an electronic signal by the instrument and is designated the "growth index". Cytotoxic effects are calculated by comparing the growth-index values of drug-treated samples to those of triplicate control vials.

Statistical considerations. ID 90 (1 h) values (i.e., the drug concentration required to reduce the growth index to 10% of control) were determined from the log-dose survival curves. The specific antitumor activity of a compound was then defined by a ratio of the ID 90 to 1/10 of clinically achievable peak plasma concentrations in man. Because of a lack of accurate pharmacokinetic data in humans for several investigational compounds being tested (didemnin B, DUP-785, flavone-8-acetic acid, fostriecin, nafidimide), an estimate of in vivo achievable PPCs (PPC') was substituted for calculating the drug-activity ratios. PPC' values were obtained by use of a multiple linear regression model that was based on a correlation between the logarithms of i.p. LD 50 values in nontumor-bearing mice and known peak plasma concentrations achievable in vivo, as previously described [19].

Results

The antitumor activity of the six compounds with known peak plasma concentrations achievable in vivo that were tested in this series is summarized in Table 1. According to our original definition of an ID 90 to 1/10 of the PPC ratio of less than 30 as a criterion for in vitro drug activity, five of the compounds, including the three recombinant interferon types, appeared to be inactive. Only trimetrexate revealed a significant inhibition of cellular growth in all three cell lines quantitatively comparable with the results obtained for the 5-fluorouracil control cultures.

The LD 50 values, the interpolated PPCs (PPC'), and the standard error of estimate (90% confidence limit) for the investigational compounds, without human pharmacokinetic data information included in this test series, are listed in Table 2. The latter values were obtained by use of a statistical regression model based on the observed correlation between acute animal toxicology data in mice (i.p. LD 50 values) and clinically achievable plasma concentrations in man. (In a retrospective analysis of the known data pairs of 28 commonly used anticancer drugs [19], we have recently shown that this model system will permit a rough estimate of PPC values for a new investigational compound with known LD 50 values in mice.) Evaluation of the ID 90 to 1/10 PPC' ratios yielded in vitro activity of DUP-785 in two of the cell lines tested; didemnin B and flavone-8 acetic acid were active only in Ht-29 cells (ID 90 to 1/10 PPC': 3.46 and 22.63, respectively). No in vitro response was obtained for nafidimide or for the novel antitumor antibiotic fostriecin (CI-920). With regard to the latter drug, it was interesting to note that in two of the human colorectal cancer cell lines, an inverse dose response effect was observed (data not shown). The finding of a lesser degree of activity at higher drug concentrations (which did

Table 2. In vitro activity of the compounds without accurate pharmacokinetic information in humans being tested in this series[a]

Drug	I.p. LD 50s in normal mice (mg/ml)	Interpolated PPC in man (μg/ml)	ID 90 to 1/10 PPC'[b]		
			COLO 320DM	OM-1	Ht-29
Didemnin B	1.7	0.25 (0.03–2.3)	243.2 (26.0–2027)	33.6 (3.6–280)	3.6 (0.4–30)
DUP-785	145.0	6.9 (1.2–56.7)	14.0 (1.6–80.5)	23.8 (2.8–137)	236.6 (26–1376)
Flavone-8-aa.	392.0	14.8 (1.7–125.9)	37.19 (4.4–316.1)	177.8 (20.9–1511)	22.6 (2.7–192.4)
Nafidimide	67.1	3.9 (0.5–32.4)	53.5 (6.4–443.0)	57.9 (6.9–479.1)	41.4 (5.0–342.3)
Fostriecin	61.1	4.5 (0.5–37.1)	1687 (203–13 967)	–	–

[a] Values indicated for each drug (and each cell line) were obtained by calculating the ratio of the ID 90 versus 1/10 of the estimate of clinically achievable peak plasma concentrations in man (PPC'). The values in parentheses define the 90% confidence limit of the ID 90 to 1/10 PPC' ratio

[b] A ratio of <30 was defined as criterion for in vitro drug activity

not allow calculation of a reliable ID 90 value) is in agreement with previous studies of the compound in a human tumor-cloning system [17].

Discussion

The majority of currently available antineoplastic drugs are very efficient against rapidly proliferating tumors (e.g., hematopoietic malignancies), but show a dismal performance against most human solid tumors. Thus, the search for new, more effective antitumor drugs will continue as will the search for an optimal preclinical screen for the multitude of agents with presumed antiproliferative activity. In response to increasing criticism concerning the adequacy and the economic aspects of the in vivo murine transplantable system currently in use, several promising new techniques have been evaluated. However, even the usefulness of the most promising techniques, such as the human tumor-cloning assay [20] and the xenografting of human tumors into athymic mice [15] for drug screening purposes, remains uncertain due to technical and financial disadvantages.

Alternatively, established human tumor cell lines have been suggested as an economic and relevant primary screening system for new anticancer compounds [2, 3]. We have recently investigated the usefulness of continuous colorectal cancer cell lines for determining the activity of antineoplastic drugs in cancer of the large bowel [18]. Based on the in vitro chemotherapeutic responsiveness to a variety of established anticancer compounds with or without known clinical activity, we have selected three cell lines that yielded clinically relevant information in terms of drug-sensitivity patterns in humans. The present study, in which several new investigational compounds were tested, represents an evaluation of the predictive potential of this screening system. The clinical relevance of our findings will only be shown with prospective phase II clinical trials in man. This concerns in particular the observed antitumor potential of dideminin B, DUP-785, and flavone-8-acetic acid since, in accordance with the lack of pharmacokinetic information on their use in humans, the prediction of the in vivo effectiveness of these drugs in colorectal cancer was based on estimated peak plasma concentration values.

The finding that the three recombinant interferon types tested lack in vitro activity may have to be interpreted critically, although interferons have only shown a minimal degree of clinical effectiveness in preliminary studies in cancer of the large bowel [6]. Since the antitumor effect of interferons might in part be mediated indirectly via their immunomodulating properties, the lack of in vitro efficacy observed could be related to the inadequacy of the present assay system for the screening of biological response modifiers. In addition, it is unknown whether the quantification of IF effects, in relation to the relatively low clinically achievable peak plasma concentrations, together with the use of 1-h (rather than a prolonged) drug exposure, will truly reflect the therapeutic situation in humans.

The lack of a clear-cut dose-response effect of the novel antitumor antibiotic fostriecin (CI-920) in two of the cell lines is not clearly understood; however, this observation is in accordance with previous findings in a human tumor-cloning assay [17]. Because of the water solubility of the substance and the quality control that has been applied to both techniques (including a positive chromomycin A3 control), a technical failure in the performance of the assay(s) is not likely to account for the finding. Although there is also no definitive biochemical explanation available at present, it may be that the known (irreversible) interaction of the compound with its own cellular uptake mechanisms [5] may account for this phenomenon.

In summary, the potential role of the Bactec system for drug screening in colorectal cancer should definitely be established in clinical phase II studies. These studies will also clarify the potential of the statistical model that has been used to predict the magnitude of peak plasma concentrations achievable in vivo for some of the investigation compounds tested in this series. According to an overall standard deviation of a predicted log PPC of 0.69 [19], this system only permitted a rough estimate of clinically achievable drug concentrations in man. However, with respect to the considerable variation in such values between various agents in humans (approximately 4 logs),

the system may prove useful for interpreting the true significance of the antitumor activity of a new compound in vitro.

References

1. Donehower RC, Graham ML, Thompson GE, Dole GB, Ettinger DS (1985) Phase I and pharmacokinetic study of trimetrexate (TMTX) in patients with advanced cancer. Proc Am Soc Clin Oncol 4: 32
2. Drewinko B (1983) Use of human malignant cell lines in screening. In: Blanka LJ, Osieka R, Schabel FM (eds) 13th International Congress of Chemotherapy, Vienna [Suppl SS 82] vol 223, pp 1–7
3. Finlay GJ, Baguley BC (1984) The use of human cancer cell lines as a primary screening system for antineoplastic compounds. Eur J Cancer Clin Oncol 20: 947–954
4. Fogh J, Trempe G (1975) New human tumor cell lines. In: Fogh J (ed) Human tumor cells in vitro. Plenum Press, New York, pp 115–159
5. Fry DW, Besserer JA, Boritzki TJ (1984) Transport of the antitumor antibiotic CI-920 into L1210 leukemia cells by the reduced folate carrier system. Cancer Res 44: 3366–3370
6. Goldstein D, Laszlo J (1986) Interferon therapy in cancer: from imagination to interferon. Cancer Res 46: 4315–4322
7. Greenberg S, Harmon M, Couch R (1980) Exogenous interferon: stability and pharmacokinetics. In: Stringfellow DA (ed) Interferon and interferon inducers. Clinical Application. Marcel Dekker, New York, pp 55–87
8. Gutterman JU, Rosenblum MG, Rios A (1984) Pharmacokinetic study of partially pure gamma-interferon in cancer patients. Cancer Res 44: 4164–4171
9. Von Hoff DD, Forseth B, Warfel LE (1984) Comparison of a radiometric (BACTEC) and a cloning system for screening for new antineoplastic agents. Proc Am Soc Clin Oncol 3: 28
10. Von Hoff DD, Forseth B, Warfel LE (1985) Use of a radiometric system to screen for antineoplastic agents: correlation with a human tumor cloning system. Cancer Res 45: 4032–4038
11. Kurnick NB, Coats HA, De Jesus I (1983) A new method for in vitro chemosensitivity assay: inhibition of metabolic CO_2 production. Biomed Pharmacother 37: 351–353
12. Long HJ, Powis G, Schutt AJ, Moertel CG, Kovach JS (1985) Menogaril by 72-hour continuous intravenous infusion: a phase I study with pharmacokinetics. Proc Am Soc Clin Oncol 4: 28
13. McPherson TA, Tan YH (1980) Phase I pharmacotoxicology study of human fibroblast interferon in human cancers. J Natl Cancer Inst 65: 75–79
14. Nelson RL, Root MA (1983) Clinical pharmacokinetics of vinzolidine, a new orally active vincaalkaloid derivate. Proc Am Soc Clin Oncol 2: 19
15. Ovejera AA, Houchens DP (1981) Human tumor xenografts in athymic nude mice as a preclinical screen for anticancer agents. Semin Oncol 8: 386–393
16. Quinn LA, Moore GE, Morgan RT, Woods LK (1979) Cell lines from human colon carcinoma with unusual cell products, double minutes, and homogeneously staining regions. Cancer Res 39: 4914–4924
17. Scheithauer W, Von Hoff DD, Clark GM, Shillis JL, Elslager EF (1986) In vitro activity of the novel antitumor antibiotic fostriecin (CI-920) in a human tumor cloning assay. Eur J Cancer Clin Oncol 22: 921–926
18. Scheithauer W, Clark GM, Moyer MP, Von Hoff DD (1986) New screening system for selection of anticancer drugs for treatment of human colorectal cancer. Cancer Res 46: 2703–2708
19. Scheithauer W, Clark GM, Salmon SE, Dorda W, Shoemaker RH, Von Hoff DD (1986) A model for estimation of clinically achievable peak plasma concentrations for investigational anticancer agents in man based on acute animal toxicology data. Cancer Treat Rep 70: 1379–1382
20. Shoemaker RH, Wolpert-Defilipes MK, Kern DH, Leiber MM, Makuch RW, Melnick NR, Miller WT, Salmon SE, Simon R, Venditti JM, Von Hoff DD (1985) Application of a human tumor colony-forming assay to new drug screening. Cancer Res 45: 2145–2153
21. Spremulli EN, Scott C, Champbell DE, Libbey NP, Schochat D, Gold DV, Dexter DL (1983) Characterization of two metastatic subpopulations originating from a single human colon carcinoma. Cancer Res 43: 3828–3835

Received May 6, 1987/Accepted September 27, 1987