Cancer Chemother Pharmacol (1988) 22: 41–46

Cancer Chemotherapy and Pharmacology

Comparison of cellular basis of drug sensitivity of human colon, pancreatic, and renal carcinoma cell lines with that of leukemia cell lines*

Shogo Ozawa, Tetsuya Yasuda, and Makoto Inaba

Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, Kami-Ikebukuro, Toshima-ku, Tokyo 170, Japan

Summary. In an attempt to find how much the low therapeutic effectiveness of antitumor drugs against so-called chemotherapy-refractory tumors such as colon carcinoma depends on drug sensitivity at the cellular level, sensitivity of five carcinoma cell lines (three colorectal, one pancreatic, and one renal) to nine typical anticancer agents was compared in vitro with that of four generally chemotherapy-susceptible leukemia cell lines. Sensitivity was assesed in terms of the percentage cell growth in control cultures, which was determined by exposing exponentially growing cells for 48 h to the following antitumor drugs: 1-(4-amino-2-methylpyridine-5-yl)-methyl-3-(2-chloroethyl)3-nitrosourea hydrochloride (ACNU), adriamycin (ADM), bleomycin (BLM), cisplatin (DDP), etoposide (VP-16), 5-fluorouracil (5FU), mitomycin C (MMC), methotrexate (MTX), and vinblastine (VLB). As expected, 10-fold or greater differences in sensitivity were scarcely ever observed between the two kinds of cell lines. Thus, we recorded a result of more (or less) sensitivity when there was a difference of 3-fold or more; and compared the drug sensitivity in every pair of carcinoma and leukemia cell lines (20 pairs for each drug). We found that carcinoma cell lines were less sensitive to VP-16, ADM, DDP, and MTX than leukemia cell lines in 18, 15, 12, and 10 of 20 pairs, respectively; only one opposite case was observed, with DDP. On the other hand, no such tendency between the two groups was observed with BLM, 5FU, or MMC. Overall, significantly different sensitivities were observed between them in 91 out of 180 pairs (i.e., 9 antitumor drugs × 5 carcinomas × 4 leukemias), and carcinoma cell lines were less sensitive than leukemia cell lines in 79 of these 91 pairs. These results suggest that the refractoriness of colon carcinoma, etc. to chemotherapy is, at least in part, due to low drug sensitivity of the tumor cell itself.

Introduction

In spite of recent advances in cancer chemotherapy, the chemotherapy of solid tumors, particularly colon, pancreatic, renal carcinoma, non-small cell carcinoma of lung, etc., remains a major clinical problem. It would be important to know what makes such cancers so refractory to chemotherapy.

In this connection, van Putten et al. [11] reviewed various mechanisms proposed for the poor responsiveness of colon tumors to chemotherapeutic agents. They made an inventory of the data available and the work that needed to be done to elucidate the mechanism(s) responsible for the low efficacy of anticancer drugs against colon tumors. They listed the following possible explanations: (a) inherent cellular insensitivity to drugs; (b) heterogeneity of response; (c) impairment of drug penetration owing to poor vascularization; (d) emergence of resistant tumor cell lines; and (e) rapid repopulation of surviving tumor cells during treatment. When we consider that such tumors seldom show definite responses even to the initial chemotherapy, (a) and (c) seem to be the most plausible causes of their refractoriness to chemotherapy.

With respect to (c), Mäntylä examined regional blood flow in lymphomas and various kinds of carcinomas, including those of the lung, breast, prostate, colon, stomach, and uterine cervix, and concluded that lymphomas possess statistically higher blood flow than these carcinomas [9]. However, no significant difference in blood flow has been reported among various kinds of carcinomas with generally different degrees of drug sensitivity. This might suggest that vascularization does not make a major contribution to the refractoriness of colon and other tumors to chemotherapy.

In the present study, therefore, using cell lines derived from these human solid tumors, and leukemia cell lines as reference lines representing sensitive tumors, we investigated the cellular level of drug sensitivity of each line. The sensitivity of five (3 colon, 1 pancreatic, and 1 renal) carcinoma cell lines to nine typical antitumor drugs was compared with that of four leukemia cell lines by the same assay method. That is, cells were exposed to two different concentrations of each drug for 48 h during their exponential growth phase, and cell numbers were determined by means of a Coulter counter. Finlay et al. [5] have reported that there is good correlation among three assay methods, namely, MTT (3,4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide), methylene blue staining, and cell counting [5]. The drug concentrations were selected to induce differential cytotoxicities among these cell lines.

Materials and methods

Cells and culture conditions. The human cancer cell lines used in this study and their sources are listed in Table 1.

* This study was supported in part by Grants-in-Aid for Cancer Research from the Ministry of Education, Science and Culture, Japan

Offprint requests to: Makoto Inaba

Table 1. Human cancer lines and their characteristics

Cell line	Histological type	Ref.	Medium	Doubling time (h)	Abbreviation
DLD-1	Colon adenocarcinoma	[4]	RPMI 1640 + 10% FCS	19.8	
LS 180	Colon adenocarcinoma	[15]	MEM + 10% FCS	19.4	
WiDr	Colon adenocarcinoma	[10]	MEM + 10% FCS	17.3	
MIA PaCa-2	Pancreatic carcinoma	[19]	MEM + 10% FCS	18.0	PaCa-2
ku-2	Renal carcinoma		MEM + 10% FCS	15.0	
CCRF-SB	Acute lymphoblastic leukemia (B cell)	[1]	RPMI 1640 + 10% FCS	23.3	SB
CCRF-CEM	Acute lymphoblastic leukemia (T cell)	[6]	RPMI 1640 + 10% FCS	19.2	CEM
CCRF-HSB-2	Acute lymphoblastic leukemia (T cell)	[2]	RPMI 1640 + 10% FCS	24.4	HSB-2
K-562	Chronic myelogenous leukemia	[8]	RPMI 1640 + 10% FCS	14.4	K562

Culture medium for the cell lines and their doubling times are also described there. All the cell lines except ku-2 were obtained from ATCC (American Type Culture Collection).

Ku-2 cells were kindly provided by Dr Tazaki, Department of Urology, School of Medicine, Keio University. The cells were established from a lung metastasis of renal cancer in a male patient. In detail, this metastatic lung tumor had been resected and transplanted to nude mice. Erythrocytosis was found in the implanted nude mice and the cells were histologically identified to be renal tumor cells. Renal cell carcinoma line termed ku-2 was established in vitro from this transplanted tumor.

All the cells were cultured in medium containing 100 μg/ml kanamycin at 37° C in the humidified atmosphere of 5% CO_2 and 95% air.

Cytotoxic drugs. The drugs used were as follows: ACNU (Sankyo, Tokyo), ADM (Kyowa Hakko Kogyo, Tokyo), BLM (Nippon Kayaku, Tokyo), DDP (Bristol Banyu, Tokyo), 5FU (Kyowa Hakko Kogyo), MMC (Kyowa Hakko Kogyo), MTX (Lederle Japan, Tokyo), VLB (Shionogi, Osaka), and VP16 (Nippon Kayaku).

For stock solutions drugs were dissolved in physiological saline at a concentration of 2 m*M*, or 6 m*M* for ACNU, except for VP16. This drug was first dissolved in a small volume of dimethylsulfoxide before the addition of sufficient physiological saline to bring the VP16 concentration to 2 m*M*; the stock solution did not contain more than 10% dimethylsulfoxide. All drug solutions were diluted with culture medium and added to the culture at the desired concentrations.

Doubling time for each cell line. Carcinoma cells (1×10^5) and leukemia cells (5×10^4) were seeded into 60-mm plates in 5 ml culture medium and into test tubes containing 1 ml culture medium, respectively. The doubling time for each cell line was determined by counting the cultured cells in triplicate every 24 h for 4 or 5 days. Carcinoma cells were trypsinized and enumerated. Cell number was determined with a Coulter counter model ZBI (Coulter Electronics).

In vitro growth inhibition assays. The drugs sensitivity of each cell line was described in terms of the percentage growth compared with control cells after exposure of exponentially growing cells to a drug at two given concentrations for 48 h.

Leukemia cells were suspended at a cell density of 5×10^4 cells/ml in culture medium containing the desired antitumor agent and incubated in a CO_2 incubator at 37° C for 48 h. Carcinoma cells were plated at a cell density of 2×10^4 cells in 35-mm plastic dishes containing 2.0 ml culture medium. The cells were incubated for 1 day to allow entry into the exponential growth phase. Each antitumor drug was added at two concentrations to separate cultures 1 day after seeding, and then the cells were incubated for a further 48 h as in the case of leukemia cells. Using additional dishes, we also determined the initial cell numbers on the day of addition of drug.

At the end of the incubation period, the number of cells was determined in a model ZBI Coulter counter. Carcinoma cells were detached from the dishes by treatment with 0.05% trypsin–0.02% EDTA solution (GIBCO).

The percentage growth was calculated as follows:

$$\% \text{ Growth} = \frac{\text{Number of cells in treated culture} - \text{Initial cell number}}{\text{Number of cells in untreated culture} - \text{Initial cell number}} \times 100\ (\%)$$

The assays were performed in triplicate and on three separate occasions. In addition, for estimation of differences in drug sensitivity between a given carcinoma and leukemia cell line, the IC_{50} (the 50% inhibitory concentration), or the inhibitory concentration for other percentages than 50% if IC_{50} was not available, was derived from the percentage growth mentioned above.

Results

Growth rate of cells

Doubling times (h) of the cell lines during the exponential growth phase and their histological types are shown in Table 1. Doubling times varied considerably from cell line to cell line.

Colon carcinoma as a tumor mass is usually characterized by slow growth in vivo. However, the doubling times of colon cancer cell lines in vitro were not necessarily long.

In vitro drug sensitivity of the cell lines

The percentages of the growth seen in control cultures for all the cell lines examined for drug sensitivity in this study are listed in Table 2. The experimental cultures had been exposed singly to each drug for 48 h.

Two drug concentrations were selected for these assays so that growth of the CEM leukemic cells would be almost

Table 2. Growth of the cell lines (% of control growth) after treatment with antitumor drugs

Compound (μM)		Carcinoma cells					Leukemic cells			
		Colon			Pancreatic MIA PaCa-2 (PaCa-2)	Renal ku-2	B-ALL CCRF-SB (SB)	T-ALL		Myelogenous K-562 (K562)
		DLD-1	LS 180	WiDr				CCRF-CEM (CEM)	CCRF-HSB-2 (HSB-2)	
ACNU	300	17± 6	10± 5	2± 0	2± 5	−10± 4	−17± 8	10± 5	−28± 4	2± 2
	30	71± 5	72± 5	72± 3	86±11	10± 2	−3± 5	87± 6	57± 3	37± 3
ADM	0.1	18±12	27± 3	7± 3	6± 6	1± 7	−18± 2	1± 1	−16±16	1± 1
	0.01	89± 9	84± 9	72± 4	90± 5	67± 8	21±11	54± 7	15±14	38± 3
BLM	10	1± 6	14± 2	80± 8	0± 5	1± 3	−17± 1	3± 1	−13±11	6± 3
	1	11± 4	36± 8	98± 3	8± 5	65±10	16± 4	57±13	−5± 6	58±10
DDP	10	5± 8	29± 3	6± 7	7± 4	−15± 2	−23± 9	8± 2	−36± 3	2± 2
	1	55±15	63± 8	55± 2	61± 9	25± 1	27± 4	56± 2	21± 4	26± 2
5FU	100	24± 3	25± 2	6± 6	21± 4	21± 5	−16± 7	8± 1	−24± 4	26± 2
	10	50± 3	59±16	23± 8	48± 8	48± 8	−10± 3	49± 1	57± 6	87± 7
MMC	1	20± 8	29±14	13± 4	10± 5	23± 5	−17± 2	15± 1	6± 1	10± 1
	0.1	52±10	47± 4	31± 9	42± 8	57± 6	21± 4	68± 2	73± 3	44± 4
MTX	0.03	44± 6	58± 7	26± 3	39± 7	81±13	7± 1	3± 1	58± 3	5± 1
	0.003	88±12	91± 9	99±11	97± 6	91± 8	97± 5	65± 9	92± 4	64± 2
VLB	0.003	45± 7	33± 8	−21±11	−1± 2	35± 4	−16±10	1±10	−19± 7	3± 2
	0.0003	88±10	82± 4	90±13	98±12	92±14	74± 6	89± 6	84± 4	69±10
VP16	1	33± 8	40± 4	43± 8	15± 7	21± 8	−20± 3	−1± 2	−33± 5	2± 2
	0.1	85±15	86±16	99± 6	88±12	77±14	14± 6	55±10	1± 4	49± 4

completely inhibited at the higher concentration and partially inhibited at the other, lower, concentration. The data listed in Table 2 are also illustrated in Figs. 1–3 according to the extent of difference in sensitivity between carcinoma and leukemia cell lines for each of the nine antitumor agents:

1. VP16. At concentrations of both 0.1 and 1 μM, all the carcinoma cell lines seemed less sensitive than leukemia cell lines (Fig. 1). In particular, the carcinoma cell lines were all clearly 10 or more times as resistant as HSB-2 and SB cell lines. We regarded a cell line whose IC_{50} was 3 or more times that of a reference cell line as "resistant". The IC_{50}'s of CEM and K562 were 0.12 and 0.096 μM, respectively. On the other hand, since the IC_{50}s of ku-2 and PaCa-2 were 0.30 and 0.33 μM, respectively, ku-2 and PaCa-2 lines were not regarded as "more resistant or less sensitive" than the CEM cell line, but they were "more resistant" than the K562 cell line. The IC_{50} in the DLD-1 cell line was 0.47 μM and those of LS 180 and WiDr cell lines were more than 0.47 μM, since LS 180 and WiDr cells were apparently less sensitive than DLD-1 cells. Therefore, DLD-1, LS180, and WiDr cell lines were all regarded as less sensitive than any of the 4 leukemia cell lines. The sen-

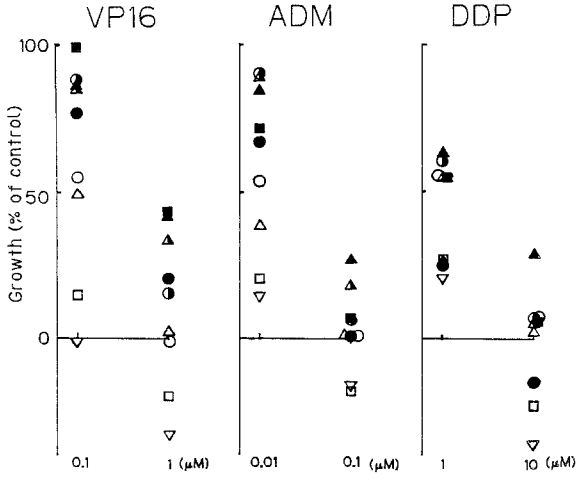

Fig. 1. Comparison of sensitivity of five carcinoma and four leukemia cell lines to VP16, ADM, and DDP. Cells were cultured with two concentrations (μM) of each drug for 48 h and the cell numbers were counted by means of a Coulter counter. Symbols and the corresponding cell lines are as follows: ▲, DLD-1; ▲, LS180; ■, WiDr; ◑, PaCa-2; ●, ku-2; □, SB; ○, CEM; ▽, HSB-2; △, K562

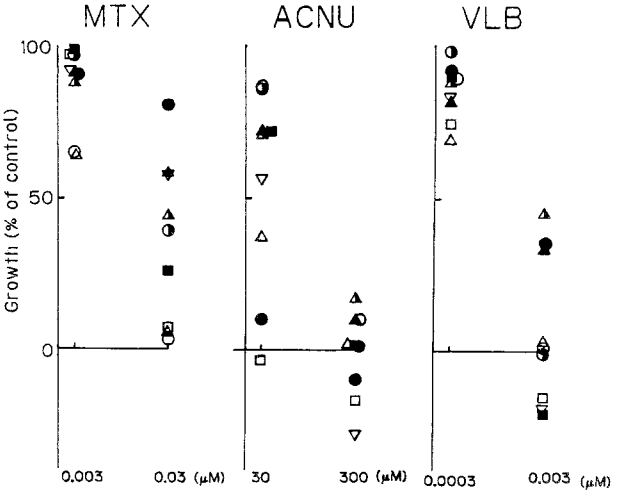

Fig. 2. Comparison of sensitivity of five carcinoma and four leukemia cell lines to MTX, ACNU, and VLB. See legend to Fig. 1 for details

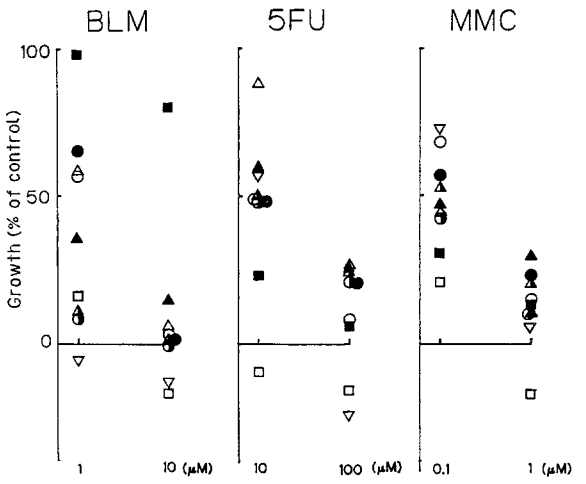

Fig. 3. Comparison of sensitivity of five carcinoma and four leukemia cell lines to BLM, 5FU, and MMC. See legend to Fig. 1 for details

sitivity of each carcinoma cell line can be compared with that of the four leukemia cell lines. Since we used five carcinoma cell lines, there were 20 pairs with one carcinoma cell line and one leukemic cell line. Regarding VP16 sensitivity, carcinoma cell lines were more highly resistant than leukemia cell lines in 18 of the 20 pairs.

2. ADM. When the growth as a percentage of control growth was compared from cell line to cell line after exposure of the cells to 0.01 μ*M* ADM, all the carcinoma cell lines seemed to be less sensitive (Fig. 1). The most highly resistant leukemia cell line was CEM. The IC_{50} for CEM cells, which was derived from the percentage growth with 0.01 and 0.1 μ*M* ADM, was 0.012 μ*M*. On the other hand, the IC_{50}s (μ*M*) of the carcinoma cell lines were 0.018 for ku-2, 0.022 for WiDr, 0.030 for PaCa-2, 0.035 for DLD-1, and 0.039 for LS 180. Therefore, only LS 180 cells were more highly resistant than CEM cells.

When K562 cells were exposed to ADM at a concentration of 0.01 μ*M*, the percentage growth of K562 cells was 38% of the control. The ADM concentrations necessary to suppress the percentage growth of the carcinoma cells to 38% were 0.027 μ*M* for ku-2, 0.034 μ*M* for WiDr, 0.042 μ*M* for PaCa-2, 0.052 μ*M* for DLD-1, and 0.064 μ*M* for LS 180. Thus, all the carcinoma cell lines but ku-2 were less sensitive than the K562 cell line. All carcinoma cell lines but ku-2 were more resistant to ADM than the HSB-2 and SB cell lines; HSB-2 and SB cell lines were apparently more sensitive than the K562 cell line. Percentage growth of HSB-2 and SB cell lines was 15% and 21%, respectively after exposure to 0.01 μ*M* ADM. The ADM concentrations necessary for reducing growth of ku-2 cells to 15% and 21% were 0.061 and 0.050 μ*M*, respectively, which means that ku-2 cells are less sensitive to ADM than HSB-2 and SB cell lines.

Therefore, in 15 of 20 pairs, carcinoma cell lines were less sensitive to ADM than leukemia cell lines.

3. DDP. At a concentration of 1 μ*M*, all the carcinoma cell lines but ku-2 seemed less sensitive than all the leukemia cell lines except for CEM (Fig. 1).

For CEM and ku-2 cells, since their growth was suppressed to 25% of control growth at 4.4 and 1.0 μ*M*, respectively, ku-2 cells were defined as "more sensitive" than CEM cells according to our criterion. Percentage growth of SB cell exposed to 1 μ*M* DDP was 27%, and DDP concentrations for reducing growth of DLD-1, WiDr, PaCa-2 and LS 180 cells to 27% were between 3.6 and 4.3 μ*M*, which showed that the carcinoma cell lines, except for ku-2, could be regarded as more highly resistant than the SB cell line. Similarly, the four carcinoma cell lines were regarded as more highly resistant than the K562 and HSB-2 cell lines.

Therefore, the four carcinoma cell lines were less sensitive than the HSB-2, K562, and SB cell lines; and ku-2 cells were more sensitive than the CEM line cells.

In 12 of the 20 pairs carcinoma cell lines were more highly resistant to DDP than leukemia cell lines, and in only 1 of 20 pairs was a carcinoma cell line more sensitive to DDP than a leukemia cell line.

4. MTX. Percentage growth of the carcinoma cell lines treated with 0.03 μ*M* MTX was greater than that of the leukemia cell lines except for the HSB-2 cell line (Fig. 2).

The IC_{50} of CEM and K562 cell lines was 0.0052 μ*M*, and they were the most sensitive of the cell lines studied. IC_{50}s of DLD-1, PaCa-2, and WiDr cell lines, which were more sensitive than ku-2 and LS 180 cell lines, were 0.022, 0.019, and 0.014 μ*M*, respectively. Therefore, CEM and K562 cell lines were three times as sensitive as any of the carcinoma cell lines, except for the WiDr cell line.

The IC_{50} of the SB cell line, which was 0.01 μ*M*, indicated that these cells were apparently at least three times as sensitive as ku-2 and LS 180 cells, because at 0.03 μ*M*, the percentage growth of ku-2 and LS 180 cell lines was more than 50%.

The percentage growth of HSB-2 cells exposed to 0.03 μ*M* MTX was 58%. The MTX concentration necessary for growth of WiDr cells to be suppressed to 58% was 0.011 μ*M*. Thus, WiDr cells were not regarded as hypersensitive to MTX compared with HSB-2 cells. Therefore, in 10 of 20 pairs, carcinoma cell lines were more highly resistant to MTX than were leukemia cell lines.

5. ACNU. Percentage growth of the carcinoma cell lines (except ku-2) exposed to 30 μ*M* ACNU was higher than that of the leukemia cell lines except in the case of CEM (Fig. 2). Four carcinoma cell lines, DLD-1, LS 180, PaCa-2, and WiDr, were markedly more resistant than SB cells. Inversely, renal carcinoma ku-2 cells, whose growth was suppressed by nearly 100% at 30 μ*M* ACNU, were 3.0 or more times as sensitive as CEM, HSB-2, and K562 cells, in which the percentage growth was suppressed to 10% at 300, 110, and 170 μ*M*, respectively. The four carcinoma cells could not be regarded as less sensitive than HSB-2 cells on the basis of either the IC_{50} or the concentration for 57% growth (i.e., the percentages found for HSB-2 cells treated with 30 μ*M* ACNU). The ACNU concentrations necessary to reduce the percentage growth of K562 cells and WiDr cells to 37% were 30 and 110 μ*M*, respectively. Thus, the four carcinoma cell lines were less sensitive than the K562 cell line, because WiDr cells were the most sensitive of the four carcinoma cells.

Therefore, in 8 of the 20 pairs carcinoma cell lines were less sensitive to ACNU than leukemia cell lines; however, in 3 of the 20 pairs, a carcinoma cell line (ku-2) was more sensitive to ACNU than leukemia cell lines.

6. VLB. At the concentration of 0.003 μ*M*, DLD-1, LS 180, and ku-2 cell lines seemed to be less sensitive than any of the leukemia cell lines (Fig. 2). However, at concentration of 0.0003 μ*M* little growth inhibition was observed in any of the cell lines studied.

The IC_{50}'s (μ*M*) for SB, CEM, HSB-2, and K562 were 0.00056, 0.00084, 0.00064, and 0.00058, respectively. On the other hand, the IC_{50}'s (μ*M*) for DLD-1, LS 180, and ku-2 were 0.0023, 0.0016, and 0.0013, respectively. Thus, we regarded DLD-1 cells as "less sensitive" than SB, HSB-2, and K562 cells.

Therefore, in only 3 out of 20 pairs was a carcinoma cell line less sensitive to VLB than the leukemia cell lines.

7. BLM. Clearly, WiDr cells were at least 10 times as resistant to BLM as any leukemia cell lines (Fig. 3). At 1 μ*M*, the percentage growth of SB cells was 16%. The percentage growth of ku-2 and LS 180 was suppressed to 16% at 5.8 and 7.8 μ*M*, respectively. HSB-2 cells were apparently more sensitive than SB cells. Therefore, ku-2 and LS 180 cells could also be regarded as less sensitive than SB and HSB-2 cell lines. On the contrary, since the percentage growth of DLD-1 was 11% at 1 μ*M* BLM, and that of CEM and K562 cells was suppressed to 11% at 7 and 8.2 μ*M*, respectively, and since PaCa-2 cells were apparently more sensitive than DLD-1 cells, DLD-1 and PaCa-2 cell lines were more sensitive than CEM and K562 cell lines.

Therefore, in 8 of the 20 pairs carcinoma cell lines were less sensitive to BLM than leukemia cell lines; however, in 4 of the 20, carcinoma cell lines were more sensitive. Two colon carcinoma cell lines (DLD-1 and LS 180) and the pancreatic cell line (PaCa-2) were as dramatically sensitive as some leukemia cell lines. Thus, there may be no clear relationship in BLM sensitivity between carcinoma and leukemia cell lines.

8. 5FU. At a concentration of 10 μ*M*, K562 cell seemed to be the least sensitive, and SB cells, the most sensitive to 5FU. All the carcinoma cell lines appeared to be about ten times or more resistant than SB cells (Fig. 3).

The IC_{50} (μ*M*) of K562 cells was 34 and those of DLD-1,PaCa-2, ku-2 and LS 180 were 10, 8.6, 8.6, and 18, respectively. The IC_{50} of WiDr cells was apparently lower than 10 μ*M*. Thus, K562 cells were less sensitive than DLD-1, PaCa-2, ku-2, and WiDr cell lines.

Therefore, in 5 of the 20 pairs, all the carcinoma cell lines were less sensitive to 5FU than one of the leukemia cell lines (SB); however, in 4 of the 20, carcinoma cell lines were more sensitive to 5FU than one of the leukemia cell lines (K562).

9. MMC. With MMC, the dose-response curve was less steep for all the cell lines, and the slope was rather different from cell line to cell line (Fig. 3).

SB cells seemed the most sensitive, whereas CEM and HSB-2 cells were the least sensitive of all the cell lines. Although there were some differences in percentage growth between some cell lines, it is difficult to determine the difference in MMC sensitivity between carcinoma and the leukemia cell lines.

In vitro anticancer drug sensitivities of various carcinoma and leukemia cell lines were thus compared. With all the pairs of carcinoma and leukemia cell lines, we determined which was likely to be three or more times as sensitive to the nine anticancer agents. The results are listed in Table 3. Overall, the carcinoma cell lines tended to be less sensitive to the antitumor agents studied than leukemia cells. Especially with VP16, ADM, DDP, and MTX, the carcinoma cell lines were less sensitive than the leukemia cell lines.

Table 3. Comparison of anticancer drug sensitivity between carcinoma and leukemia cell line

Drug	Number of cases with higher sensitivity		
	L > C	C > L	Others
VP16	18	0	2
ADM	15	0	5
DDP	12	1	7
MTX	10	0	10
ACNU	8	3	9
VLB	3	0	17
BLM	8	4	8
5FU	5	4	11
MMC	Difficult to determine		20
Total	79	12	89

L > C, a leukemia cell line (L) is more sensitive than a carcinoma cell line (C)

Discussion

It is quite important to ascertain to what extent refractoriness to chemotherapy of colon, renal, and pancreatic tumors is due to inherent drug sensitivity at the cellular level. For this purpose, in the present study, we compared the sensitivity of five established cell lines of these carcinomas to various antitumor agents with that of four generally drug-sensitive leukemia lines.

The use of cell lines in culture in such a study is always a controversial problem. It is uncertain how well an established culture line reflects the drug sensitivity characteristics of the parent tumor. Since the cell population of a tumor is generally heterogeneous [16–18], any cell lines derived may differ from the parent tumor in terms of drug sensitivity. However, the drug sensitivity of cultured cells seems to be at least within the range of drug sensitivities expressed by the parent tumor. In other words, a given cell line cannot reproduce drug sensitivity of the parent tumor exactly, but can reflect it to some extent. Thus, the use of as many cell lines as possible from a particular type of tumor may increase this probability.

The human tumor clonogenic assay using fresh tumor specimens may provide useful information in this respect. However, comparison of drug sensitivities of colon tumors, for example, with those of leukemia cells has rarely been reported, since leukemia cells seem to form few colonies with this method.

Another critical point to be discussed is the different characteristics of the growth rate of tumor cells between in vivo and in vitro conditions. It is generally believed that colon tumors, for example grow much more slowly in vivo than do leukemias, and that this is one of the major factors contributing to their refractoriness to chemotherapy. In fact, doubling times of a tumor mass in these carcinomas are tremendously long [3]. However, this is probably due to the very small fraction of the tumor that is actually in-

volved in growth. The actual cell cycle times of growing cells, for example in the colon tumor, have been reported to be not much different from those of leukemia cells [7, 14]. On the other hand, cell lines derived from such carcinomas grow rather more rapidly in culture than those of leukemias: the average in vitro doubling times of the five carcinoma and four leukemia cell lines used in this study were 17.9 and 20.4 h, respectively (Table 1). Therefore, as far as cell cycle time is concerned, there may be no great discrepancy between in vitro and in vivo growth of these tumor cells.

In any event, in spite of their rather faster growth rates, the present study has demonstrated that colon, renal, and pancreatic carcinoma cells, on the whole, show a definite tendency toward lower drug sensitivity than leukemia cells. These results might suggest that the growth rate is just one factor influencing drug sensitivity. However, many other factors, such as capacity for membrane transport, activity of enzymes involved in activating or inactivating drugs or affected by drugs, and repair capability, seem to determine the inherent sensitivity of individual tumors to a given drug. As a matter of fact, the fastest growing K562 cells were not usually highly sensitive, whereas the most slowly-growing of the cells tested, the HSB-2 cells, were considerably sensitive.

It should be noted, in particular, that the extent of difference in drug sensitivity between carcinoma and leukemia cell lines was not so great as we had expected. In practical chemotherapy for cancer patients it is impossible to increase the dose to 3 times the usual dose or more. Therefore, we decided to compare drug sensitivity between carcinoma and leukemia cell lines by the criterion of 3-fold or greater difference in effective concentrations.

Finlay et al. compared four colon carcinoma and eight leukemia cell lines for sensitivity to eight DNA-binding agents (daunorubicin, doxorubicin, mitoxantrone, amsacrine, 4-methylamsacrine, CI-921, acridine carboxamide, and nitracrine) and observed that the colon carcinoma cell lines were significantly more highly resistant to these drugs than the leukemia cell lines [5]. In the present study, colon carcinoma cell lines exhibited lower sensitivity not only to ADM, but also to VP16, DDP, and MTX, than did leukemia cell lines. On the other hand, some of the colon carcinoma cell lines were fairly sensitive to 5FU, which is one of the few drugs clinically available for the treatment of colon cancer. This result was also obtained in the study published by Finlay et al. [5].

In addition, Takahashi et al. [13] and Ruckdeschel et al. [12] compared established lines of small cell and non-small cell lung carcinomas for in vitro drug sensitivity and found that the former lines were significantly more sensitive to cisplatin and other drugs than the non-small cell lines. These results are in good agreement with those of clinical treatments, providing support for the use of in vitro sensitivity testing with established cell lines of human tumors to predict clinical outcome.

In conclusion, the present comparative study on drug sensitivity of carcinoma and leukemia cell lines suggests that poor responsiveness to chemotherapy of colon, renal, and pancreatic tumors is due to the inherent low drug sensitivity of the tumor cells themselves. We also conclude that the degree of difference in sensitivity between the two groups is not so pronounced, although further extensive studies might be needed to confirm our results. To improve chemotherapeutic effectiveness against these refractory tumors, it is particularly important to study the cellular mechanism(s) responsible for their relatively low sensitivity to some antitumor agents. With such knowledge, new treatment regimens can be developed to overcome this problem. Our current efforts are directed toward this goal.

References

1. Adams RA (1967) Formal discussion: the role of transplantation in the experimental investigation of human leukemia and lymphoma. Cancer Res 27: 2479
2. Adams RA, Flowers A, Davis BJ (1968) Direct implantation and serial transplantation of human acute lymphoblastic leukemia in hamsters. SB-2. Cancer Res 28: 1121
3. Charbit A, Mulaise EP, Tubiana M (1971) Relation between the pathological nature and the growth rate of human tumors. Eur J Cancer 7: 307
4. Dexter DL, Barbosa JA, Calabresi P (1979) *N,N*-Dimethylformamide-induced alteration of cell culture characteristics and loss of tumorigenicity in cultured human colon carcinoma cells. Cancer Res 39: 1020
5. Finlay GJ, Wilson WR, Baguley BC (1986) Comparison of in vitro activity of cytotoxic drugs towards human carcinoma and leukemia cell lines. Eur J Cancer Clin Oncol 22: 655
6. Foley GE, Lazarus H, Farber S, Uzman BG, Boone BA, McCarthy RE (1965) Continuous culture of human lymphoblasts from peripheral blood of a child with acute leukemia. Cancer 18: 522
7. Greenberg ML, Chanana AD, Cronkite EP, Giacomelli G, Rai KR, Schiffer LM, Stryckmans PA, Vincent PC (1972) The generation time of human leukemic myeloblasts. Lab Invest 26: 245
8. Lozzio CB, Lozzio BB (1975) Human chronic myelogenous leukemia cell-line with positive Philadelphia chromosome. Blood 45: 321
9. Mäntylä MJ (1979) Regional blood flow in human tumors. Cancer Res 39: 2304
10. Noguchi P, Wallace R, Johnson J, Earley EM, O'brien S, Ferrone S, Pellegrino MA, Milstien J, Needy C, Browne W, Petricciani J (1979) Characterization of WiDr: a human colon carcinoma cell line. In Vitro 15: 401
11. Putten LM van, Sluijter EA, Smink T, Mulder JH (1981) Why do colon tumours respond poorly to chemotherapeutic agents? Recent Results Cancer Res 79: 10
12. Ruckdeschel JC, Carney DN, Oie HK, Russel EK, Gazdar AF (1987) In vitro chemosensitivity of human lung cancer cell lines. Cancer Treat Rep 71: 697
13. Takahashi H, Sasaki Y, Saijo N, Sakurai M, Nakano H, Nakagawa K, Hoshi A, Jett JR, Hong W-S (1987) In vitro colony inhibition of carboplatin against stomach and lung cancer cell lines in comparison with cisplatin. Cancer Chemother Pharmacol 19: 197
14. Terz JJ, Curutchet HP, Lawrrence WJ (1971) Analysis of the cell kinetics in five human solid tumors. Cancer 28: 1100
15. Tom BH, Rutzky LP, Jakstys MM, Oyasu R, Kaye CI, Kahen BD (1976) Human colonic adenocarcinoma cells: I. Establishment and description of a new line. In vitro 12: 180
16. Tsuruo T, Fidler IJ (1981) Differences in drug sensitivity among tumor cells from parental tumors, selected variants, and spontaneous metastases. Cancer Res 41: 3058
17. Wolf CR, Hayward IP, Lawrie SS, Buckton K, McIntyre MA, Adams DJ, Lewis AD, Scott ARR, Smyth JF (1987) Cellular heterogeneity and drug resistance in two ovarian adenocarcinoma cell lines derived from a single patient.
18. Yung W-A, Shapiro JR, Shapiro WR (1982) Heterogenous chemosensitivities of subpopulations of human glioma cells in culture. Cancer Res 42: 992
19. Yunis AA, Arimura GK, Russin DJ (1977) Human pancreatic carcinoma (MIA PaCa-2) in continuous culture: sensitivity to asparaginase. Int J Cancer 19: 128

Received November 2, 1987/Accepted January 15, 1988