Cancer Chemother Pharmacol (1985) 14: 132–134

Cancer Chemotherapy and Pharmacology

Comparison of the cellular pharmacology of doxorubicin in resistant and sensitive models of pancreatic cancer

Barbara K. Chang[1] and Judith A. Gregory[2]

[1] Division of Hematology/Oncology, Department of Medicine, Medical College of Georgia and VA Medical Center, Augusta, GA 30910, USA
[2] Medical Research Service, VA Medical Center, Augusta, Georgia, USA

Summary. *The cellular accumulation and retention of doxorubicin (ADR) was investigated in two models of pancreatic adenocarcinoma, which differ markedly in their sensitivity to ADR. In vitro studies revealed that the relatively resistant cell line, WD PaCa (or well-differentiated pancreatic adenocarcinoma of the Syrian hamster), actually had higher ADR cellular levels than the sensitive cell line, PD PaCa (or poorly differentiated pancreatic adenocarcinoma of the Syrian hamster). While the efflux of ADR from WD PaCa was greater, the overall retention of ADR by WD PaCa was comparable to PD PaCa. These results failed to document differences in accumulation or retention of ADR capable of explaining the difference in the sensitivity of the cell lines to ADR and indicate the need to search beyond attainable drug concentrations for mechanisms of primary ADR resistance.*

Introduction

Animal models of adenocarcinoma of the pancreas have only recently become available through studies of pancreatic carcinogenesis [8, 13–16, 21]. Two of these models, WD PaCa and PD PaCa, in the Syrian hamster have been characterized in our laboratory with respect to their sensitivity to various chemotherapeutic agents [5, 6]. Since the two models differ greatly in their in vitro and in vivo sensitivity to doxorubicin [Adriamycin (ADR)], it was felt that examination of the cellular accumulation of ADR might shed some light on the mechanism of their difference in chemosensitivity. In addition, it was recognized that in spite of the limited chemosensitivity of human pancreatic adenocarcinoma and the need for improved therapy for this disorder [17, 20], there is a lack of published information on the handling of chemotherapeutic agents by pancreatic adenocarcinoma.

While differences in the in vitro levels of ADR were found, the findings suggest that the problem of intrinsic drug resistance/sensitivity may be the most important factor in mediation of the response of the cell lines studied to ADR.

Abbreviations: WD PaCa, well-differentiated ductal pancreatic adenocarcinoma; PD PaCa, poorly-differentiated ductal pancreatic adenocarcinoma; ADR, doxorubicin; ID_{50} and ID_{90}, drug dose in μg/ml for 1 h exposure at which there is a 50% or a 90% inhibition of survival as compared with controls

Offprint requests to: B. K. Chang

Material and methods

Tumor models. The animal models from which the cell lines used in this study were derived were supplied to us courtesy of Drs D. G. Scarpelli and M. S. Rao, Department of Pathology, Northwestern University (Chicago). WD PaCa is a carcinogen-induced solid tumor [by *N*-nitrobis (2-oxopropyl)-amine] that is passaged in Syrian hamsters and described in detail elsewhere [16]. PD PaCa is a spontaneously derived Syrian hamster tumor, which was originally developed and propagated as a solid tumor by Dr Kirkman, Department of Anatomy, Stanford University. Drs Rao and Scarpelli [13] subsequently adapted it to the ascitic form that was supplied to us.

Both tumor models have been adapted in our laboratory to in vitro growth. WD PaCa grows as an adherent monolayer and has been in continuous culture for over 2 years. PD PaCa adapts readily to tissue culture conditions and grows as a single-cell suspension. Doubling times average 24 h for WD PaCa and 18 h for PD PaCa when passaged in complete medium (RPMI-1640, supplemented with 10% heat-inactivated fetal bovine serum, glutamine, penicillin 100 units/ml and streptomycin, 100 μg/ml) GIBCO, Grand Island, NY, except serum from Flow Laboratories, McLean, Va). Both cell lines form colonies in semisolid media.

Cell counts and viability. Cell counts were performed manually and cell viability was estimated by the exclusion of trypan blue.

Drug exposure. In vitro cellular ADR concentrations were determined using the cultured cell lines harvested in late log or early stationary phase growth. WD PaCa cells were harvested by short exposure to 0.25% trypsin. Cells ($2.5–5.0 \times 10^6$ viable cells/ml) were incubated at 37° C, 5% CO_2 with ADR at concentrations of 0.1, 1.0, 10, 50, and 100 μg/ml in complete medium. After 1 h of incubation, cells were washed twice in Hanks' Balanced Salt Solution (GIBCO) and sedimented cells were processed as described below. Accumulation/retention studies were similarly performed at an ADR concentration of 10 μg/ml, with incubation and sampling times of 15, 30, 60, 75, 90, and 120 min. Samples incubated beyond 60 min were washed twice and resuspended in fresh complete medium. Each concentration or incubation point was studied in triplicate. Cell counts and viability were determined in an aliquot from the final wash of each sample. ADR content is expressed as micrograms of ADR equivalents/10^7 viable cells.

Table 1. Comparison of cellular accumulation of ADR by WD PaCa and PD PaCa following 1 h in vitro exposure to increasing concentrations of ADR

ADR concentration	Cellular content ($\bar{x}$ + SD) ($\mu g/10^7$ viable cells)	
	WD PaCa	PD PaCa
0.1 μg/ml	0.092 ± 0.010	0.204 ± 0.001*
1.0 μg/ml	1.13 ± 0.011	0.755 ± 0.04*
10.0 μg/ml	8.2 ± 0.25	4.5 ± 0.24*
50.0 μg/ml	23.5 ± 1.1	14.9 ± 0.6*
100.0 μg/ml	86.1 ± 28.1	30.5 ± 8.8**

* $P < 0.001$; ** $P < 0.05$

Drug assay. The method of Bachur et al. [3, 4] was used to measure ADR concentrations. Sedimented cells (containing 2.5–5.0 × 10^6 viable cells) were resuspended in 2 ml 0.3 *N* HCl: 50% ethanol, vortexed, disrupted by sonication, and revortexed. After centrifugation (20,000 *g* for 20 min), total fluorescence of the extracts (clear supernatants) was measured at 588 nm emission and 470 nm activation wavelength in an Aminco-Bowman spectrofluorometer. Cellular levels were determined by comparing the sample fluorescence to freshly prepared standard dilutions of ADR in 0.3 *N* HCL: 50% ethanol and to control extracts from untreated cells. The assay is sensitive to a level of 0.01 μg/g in our hands. The assay does not distinguish between ADR and its fluorescent metabolites, and therefore all references to ADR content in this report refer to ADR equivalents. Similar methods have been used by other investigators, since the kinetic behavior of ADR parallels that of total fluorescence [12]. Furthermore, analysis of ADR metabolites, studied in detail by others and reviewed by Bachur [2], was felt to be beyond the scope of the present study. Intracellular fluorescence due to ADR was verified by fluorescence microscopy of untreated (control) and ADR-treated cells. Cellular accumulation by WD PaCa and PD PaCa was compared by Student's *t*-test.

Results

The results of the concentration-dependent uptake of ADR by WD PaCa and PD PaCa are shown in Table 1. A plot of the intracellular vs the extracellular concentrations of ADR is shown in Fig. 1 and permits determination of the intracellular levels at the ID_{90} levels (extracellular concentration at which there is a 90% inhibition of colony growth compared with controls) previously reported for each cell line [6]. Surprisingly, at all concentrations except 0.1 μg/ml, WD PaCa had higher accumulation of ADR at 1 h than PD PaCa. The accumulation/retention studies at 10 μg/ml (see Fig. 2) show that WD PaCa, despite its higher initial uptake, showed poorer retention of ADR. However, after 1 h incubation in drug-free medium, WD PaCa had similar levels to PD PaCa.

Discussion

In this report, the uptake of ADR by two cell lines of pancreatic adenocarcinoma has been examined in the search for an explanation of their markedly different sensitivities to ADR. In a separate report [6] it was shown that the in vivo growth (using tumor volume as the endpoint) of WD PaCa was not inhibited by ADR as a single injection of 3.75 mg/kg or 6.0

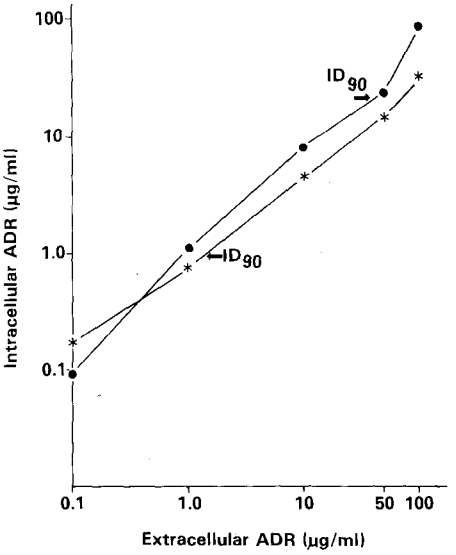

Fig. 1. Log-log plot of intracellular vs extracellular ADR concentrations in WD PaCa (●) and PD PaCa (★) following a 1-h incubation with ADR. The figures for ID_{90} are from previously reported data [6]

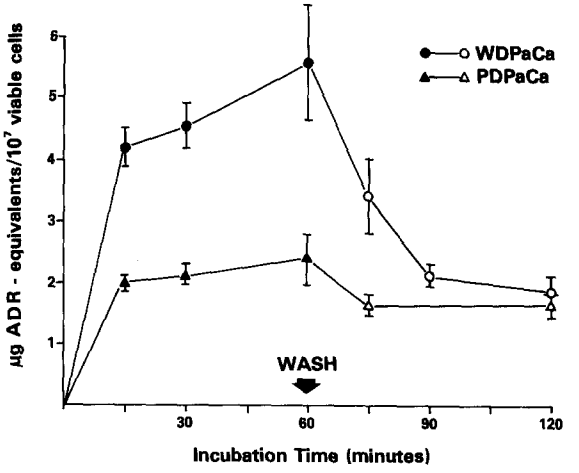

Fig. 2. In vitro comparison of the cellular accumulation *(closed symbols)* when incubated at 37° C, 5% CO_2 with 10 μg ADR/ml, and retention *(open symbols)* of ADR following incubation in drug-free medium by WD PaCa and PD PaCa

mg/kg IP (the LD_{10} and LD_{50}, respectively). PD PaCa-implanted animals (using survival as the endpoint) have a median survival of 52.5 ± 3.95 days when treated with 3.75 mg/kg ADR, as against 16.25 ± 4.15 days in controls. Likewise, in vitro clonogenic assays following a 1-h exposure to ADR confirmed the differences in sensitivity to ADR: ID_{50} = 0.45 μg/ml (0.8 μ*M*), ID_{90} = 1.6 μg/ml (2.8 μ*M*) for PD PaCa; and ID_{50} = 2.6 μg/ml (4.5 μ*M*), ID_{90} = 45 μg/ml (77.6 μ*M*) for WD PaCa. Based upon Fig. 1, the ID_{90} would correspond to an intracellular content of 1.1 μg (1.9 nmol)/10^7 cells for PD PaCa and 22 μg (38 nmol)/10^7 cells for WD PaCa. Since ADR concentration × time determinations for plasma have been reported in the range of 1.38 to 3.84 μg-h/ml [1], one can readily appreciate from the data presented that tumoricidal concentrations can be achieved in vivo for PD PaCa, but are virtually impossible to achieve for WD PaCa at doses tolerable to the host.

In vitro cellular accumulation/retention data fail to demonstrate a block to the uptake of ADR by WD PaCa cells, which show an initially greater concentration than that seen in PD PaCa cells. In retention studies, peak WD PaCa levels, however, quickly revert to ADR levels similar to those seen in PD PaCa. Since the ADR intracellular concentrations attained by WD PaCa are quite cytotoxic for PD PaCa, it is concluded that the data do *not* demonstrate differences in ADR accumulation (or efflux) that would account for the differences in sensitivity to ADR between WD PaCa and PD PaCa. Moreover, WD PaCa exhibits a pattern of generalized drug resistance in contrast to PD PaCa [5], which supports the concept of inherent phenotypic characteristics (e.g., degree of differentiation, doubling time, activity of certain enzymes, etc.) being related to drug sensitivity/resistance. Our findings in this regard are consistent with those of other investigators who feel that ADR sensitivity/resistance must be explained by other intrinsic cellular properties [7, 9, 18]. Other reports of reduced intracellular ADR [10, 19] or enhanced anthracycline efflux [11] as a mechanism of drug resistance appear not to be applicable to the pancreatic adenocarcinoma models studied. It is interesting to note that these latter reports pertain to tumor models with *acquired* resistance to ADR, in contrast to the WD PaCa model, which exhibits *primary* resistance to ADR.

Thus, drug accumulation/retention per se may be insufficient to explain differences in drug sensitivity even when there are marked differences in drug uptakes. This conclusion is somewhat disturbing, for it implies that even if the problems of drug delivery are reduced to some extent (e.g., with liposomes or selective intra-arterial infusion of drug), certain malignancies may still exhibit a biological unresponsiveness at tolerable doses. Thus, the present work indicates the need for further investigation of the molecular biology of drug-resistant cancer cells, as well as the need for more effective agents against pancreatic adenocarcinoma.

Acknowledgements. The work reported in this paper was supported by the Medical Research Service of the Veterans Administration and in part by grants nos. AM 13131 and CA 34170 awarded by the National Institutes of Health and the National Cancer Institute, DHSS.

The authors wish to thank Mary Anne Jones (VAMC, Medical Service) for secretarial support and Harry Davis (Division of Systems and Computer Services, Research and Educational Support, Medical College of Georgia) for assistance in the statistical analysis.

References

1. Alberts DS, Chen H-SG (1980) Tabular summary of pharmacokinetic parameters relevant to in vitro drug assay. In: Salmon SE (ed) Cloning of human tumor stem cells. Liss, New York, p 351
2. Bachur NR (1975) Adriamycin (NSC-123127) pharmacology. Cancer Chemother Rep 6: 153–158
3. Bachur NR, Moore AL, Bernstein JG, Liu A (1970) Tissue distribution and disposition of daunomycin (NSC82151) in mice: Fluorometric and isotopic methods. Cancer Chemother Rep 54: 89–94
4. Benjamin RS, Riggs CE, Bachur NR (1973) Pharmacokinetics and metabolism of Adriamycin in man. Clin Pharmacol Ther 14: 592–600
5. Chang BK (1983) Differential sensitivity of pancreatic adenocarcinoma cell lines to chemotherapeutic agents in culture. Cancer Treat Rep 67: 355–361
6. Chang BK, Gutman R (1982) Chemotherapy of pancreatic adenocarcinoma: Initial report on two transplantable models in the Syrian hamster. Cancer Res 42: 2666–2670
7. Chervinsky DS, Wang JJ (1976) Uptake of adriamycin and daunomycin in L1210 and human leukemia cells: A comparative study. J Med 7: 63
8. Corbett TH, Roberts BJ, Leopold WD, Peckham JC, Wilkoff LJ, Griswold DP Jr, Schabel FM Jr (1984) Induction and chemotherapeutic response of two transplantable ductal adenocarcinomas of the pancreas in C57BL/6 mice. Cancer Res 44: 717–726
9. Donelli MG, Barbieri B, Erba E, Pacciarini MA, Salmona A, Garattini S, Morasca L (1979) In vitro uptake and cytotoxicity of adriamycin in primary and metastatic Lewis lung carcinoma. Eur J Cancer 15: 1121–1128
10. Ganapathi R, Reiter W, Krishan A (1982) Intracellular adriamycin levels and cytotoxicity in adriamycin-sensitive and adriamycin-resistant P388 mouse leukemia cells. J Natl Cancer Inst 68: 1027–1032
11. Inaba M, Johnson RK (1978) Uptake and retention of adriamycin and daunorubicin by sensitive and anthracycline resistant sublines of P388 leukemia. Biochem Pharmacol 27: 2123–2130
12. Piazza E, Donelli MG, Broggini M, Sessa C, Natale N, Ottolenghi L, Marsoni S, Libretti A, Mangioni C, Morasca L (1980) Early phase pharmacokinetics of doxorubicin (adriamycin) in plasma of cancer patients during single- or multiple-drug therapy. Cancer Treat Rep 64: 845–854
13. Rao MS, Scarpelli DG (1979) Development of an ascites tumor from a solid transplantable pancreatic adenocarcinoma of the Syrian golden hamster. Presented at the Meetings of the American Pancreatic Association and the National Pancreatic Cancer Project, Chicago, November 1979
14. Reddy JK, Rao MS (1977) Transplantable pancreatic carcinoma in the rat. Science 198: 78–80
15. Sayers HJ, Dail DH, Orloff MJ (1978) An animal model of pancreatic cancer involving whole pancreas transplantation. Surg Forum 29: 122
16. Scarpelli DG, Rao MS (1979) Transplantable ductal adenocarcinoma of the Syrian hamster pancreas. Cancer Res 39: 452–458
17. Schein PS, Lavin PT, Moertel CG, Frytak S, Hahn RG, O'Connell MJ, Reitemeier RJ, Rubin J, Schutt AJ, Weiland LH, Kalser M, Barkin J, Lessner H, Mann-Kaplan R, Redlhammer D, Silverman M, Troner J, Douglass HO Jr, Milliron S, Lokich J, Brooks J, Chaffe J, Like A, Zamcheck N, Ramming K, Bateman J, Spiro H, Livstone E, Knowlton A (1978) Randomized phase II clinical trial of adriamycin, methotrexate and actinomycin-D in advanced measurable pancreatic carcinoma: a Gastrointestinal Tumor Study Group Report. Cancer 42: 19–22
18. Silvestrini R, Lenaz L, DiFronzo G, Sanfilippo O (1973) Correlations between cytotoxicity, biochemical effects, drug levels, and therapeutic effectiveness of daunomycin and adriamycin on sarcoma 180 ascites in mice. Cancer Res 33: 2954–2958
19. Skovsgaard T (1978) Mechanisms of resistance to daunorubicin in Ehrlich ascites tumor cells. Cancer Res 38: 1785–1791
20. Smith FP, Schein PS (1979) Chemotherapy of pancreatic cancer. Semin Oncol 6: 368–377
21. Townsend CM, Franklin RB, Gelder FB, Glass E, Thompson JC (1982) Development of a transplantable model of pancreatic duct adenocarcinoma. Surgery 92: 72–78

Received April 16, 1984/Accepted July 13, 1984