

# Chemotherapy of Pancreatic Adenocarcinoma: Initial Report on Two Transplantable Models in the Syrian Hamster<sup>1</sup>

Barbara K. Chang<sup>2</sup> and Robert Gutman

Division of Hematology/Oncology, Department of Medicine, Medical College of Georgia, Augusta, Georgia 30912, and Oncology Research, Medical Research Service, VA Medical Center, Augusta, Georgia 30910

## ABSTRACT

Experimental evaluation of chemotherapy of pancreatic cancer has been limited by the lack of suitable animal models, which have only recently become available. The present study is the first report on the chemosensitivity of two transplantable animal models of pancreatic adenocarcinoma. The single-agent antitumor activity of 5-fluorouracil, cyclophosphamide, mitomycin C (MMC), methotrexate, actinomycin D, vincristine, and two dose levels of Adriamycin (ADR) were tested against established palpable tumors of well-differentiated pancreatic ductal adenocarcinoma (WD PaCa), a solid tumor model of the Syrian hamster. None of the agents or dosages of ADR were effective against palpable WD PaCa tumors. ADR, MMC, streptozotocin, and the combination of 5-fluorouracil, ADR, and MMC were similarly ineffective when administered 1 week after WD PaCa implantation, while tumors were still nonpalpable. The behavior of poorly differentiated pancreatic ductal adenocarcinoma (PD PaCa), an ascitic model of the Syrian hamster, was studied for comparison. *In vivo*, with survival as the end point, PD PaCa is markedly sensitive to ADR, perhaps weakly sensitive to MMC, and resistant to streptozotocin. *In vitro* clonogenic assays from cultured PD PaCa and WD PaCa confirmed the pattern of response seen *in vivo*. The data suggest that these recently developed pancreatic cancer models can be profitably used and compared, both *in vivo* and *in vitro*, as examples of relatively chemotherapy resistant (WD PaCa) and more sensitive (PD PaCa) tumor models.

## INTRODUCTION

Progress in the chemotherapy of pancreatic adenocarcinoma has been hampered by the lack of appropriate animal tumor models, which have only recently become available through studies of pancreatic carcinogenesis (8, 10, 11).<sup>3</sup> While several models have been described, the models developed by Rao and Scarpelli (11)<sup>3</sup> of Northwestern University have several features which make them more suitable for testing the antitumor activity of cytotoxic agents. Both the WD PaCa<sup>4</sup> and PD

PaCa are readily transplantable models with stable growth patterns; also, both models are ductal adenocarcinomas and therefore histologically similar to 75% of all human nonendocrine pancreatic cancers (4). Prior to the study reported herein, neither of these models had been tested for its sensitivity to chemotherapeutic agents.

The aim of the present study was to determine the single-agent activity against the WD PaCa and PD PaCa of a number of drugs commonly used in clinical oncology, including drugs thought to have some activity against human pancreatic adenocarcinoma (*i.e.*, 5-fluorouracil, MMC, SZN, and ADR) and some not adequately tested (*i.e.*, actinomycin D, methotrexate, cyclophosphamide, and vincristine). In addition, FAM was tested against the WD PaCa, since this combination has the best reported activity against human pancreatic adenocarcinoma (1, 12). ADR was focused upon because of our own work showing an extremely high uptake of ADR in the animal pancreas (2, 3).

## MATERIALS AND METHODS

### Animals

Normal male LHC or LSH Syrian hamsters (7 to 12 weeks old) (Charles River Breeding Laboratories, Newfield, N. J.) were used in all experiments. Animals were housed in metal cages in groups of 3 to 5 and given food and water *ad libitum*.

### *In Vivo* Tumor Models

The WD PaCa and PD PaCa models used in this study were developed and supplied to us through the courtesy of Drs. D. G. Scarpelli and M. S. Rao, Department of Pathology, Northwestern University. The WD PaCa was originally induced in LHC Syrian hamsters by weekly s.c. injections of *N*-nitrobis(2-oxopropyl)amine in 0.9% NaCl solution (10 mg/kg/week) for 10 weeks. Subsequently, the tumor was serially passaged every 8 to 10 weeks by s.c. trocar implantation in LSH or LHC inbred Syrian hamsters. It was characterized as a well-differentiated pancreatic ductal adenocarcinoma and described in detail elsewhere (11). At the time of the reported experiments, WD PaCa was in its 16th passage.

Although the PD PaCa is also a hamster pancreatic ductal adenocarcinoma, it differs from the WD PaCa by being spontaneously derived and poorly differentiated. Originally, Dr. Kirkman of the Department of Anatomy, Stanford University, propagated PD PaCa as a solid tumor, but Drs. Rao and Scarpelli subsequently adapted it to the ascitic form that we used in our experiments.<sup>3</sup> PD PaCa was maintained in the above hamster strains by i.p. injection of 2 to 3 × 10<sup>6</sup> cells once a week.

### *In Vitro* Tumor Models

Prior to its use in assays, WD PaCa was cultivated for 8 months as an adherent monolayer. When used as a source of cells for assays, cultures were free of fibroblasts or other contaminating cells. PD PaCa

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<sup>2</sup> To whom requests for reprints should be addressed, at the Hematology/Oncology Section (111E), VA Medical Center, Augusta, Ga. 30910.

<sup>3</sup> M. S. Rao and D. G. Scarpelli. Development of an ascites tumor from a solid transplantable pancreatic adenocarcinoma of the Syrian golden hamster. Joint Meeting of the American Pancreatic Association and National Pancreatic Cancer Project, Chicago, Ill., November 1979, submitted for publication.

<sup>4</sup> The abbreviations used are: WD PaCa, well-differentiated ductal pancreatic adenocarcinoma; PD PaCa, poorly differentiated ductal pancreatic adenocarcinoma; MMC, mitomycin C; SZN, streptozotocin; ADR, doxorubicin (Adriamycin); FAM, 5-fluorouracil, ADR, and MMC; LD<sub>10</sub>, 10% lethal dose; LD<sub>50</sub>, 50% lethal dose.

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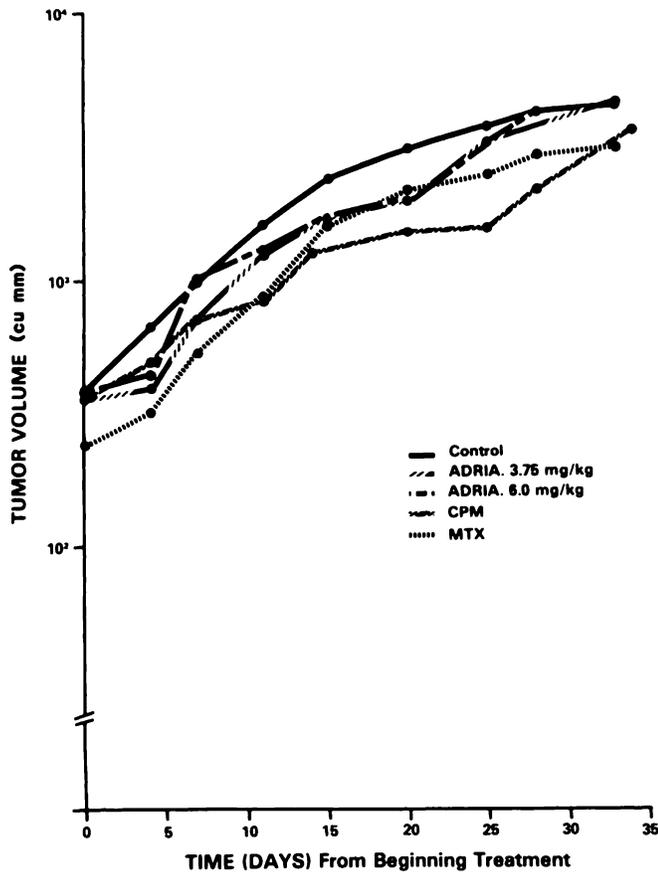


Chart 1. WD PaCa tumor growth curves following i.p. treatment with ADRIA (ADRIA; 3.75 mg/kg on Day 1 and 6.0 mg/kg on Day 1), cyclophosphamide (CPM; 150 mg/kg on Days 1 and 8), and methotrexate (MTX; 1.8 mg/kg on Days 1 and 8). No significant differences between any treated group (8 to 9 animals/group) and the untreated controls (21 animals) were seen.

adapted readily to culture conditions, growing as a single-cell suspension. Both cell lines have an approximate doubling time of 36 hr. WD PaCa and PD PaCa were passaged and maintained in complete medium, *i.e.*, Roswell Park Memorial Institute Tissue Culture Medium 1640, supplemented with 10% heat-inactivated fetal bovine serum, glutamine, penicillin (100 units/ml), and streptomycin (100  $\mu$ g/ml) (Grand Island Biological Co., Grand Island, N. Y.).

#### Cell Counts and Viability

Cell concentrations were determined manually, and their viability was determined by trypan blue exclusion.

#### Antitumor Agents

Drug dosages and schedules of administration are detailed in Charts 1 to 5. Dosages were derived from published data on the hamster or estimated from rat data and were at the LD<sub>10</sub> level, which is considered the maximum tolerated dose in animal systems (6). Dose levels of ADR, MMC, SZN, and FAM were determined by preliminary toxicological evaluation (since reliable hamster data did not exist). The 2 dose levels of ADR represent the LD<sub>10</sub> (3.75 mg/kg i.p., Day 1) and the LD<sub>50</sub> (6.0 mg/kg i.p., Day 1) levels.

#### Experiments

**In Vivo Testing Using Palpable WD PaCa Tumors.** Uniform fragments of WD PaCa approximately 1 cu mm were implanted with sterile trocars into the shoulder region of 100 hamsters, and treatment began

when the tumor volumes reached an average of about 300 cu mm (4 weeks after implantation).

The animals were weighed and tumors were measured 2 to 3 times/week with vernier calipers in 3 dimensions to the nearest 0.5 mm. Tumor volumes were calculated, with no correction for skin thickness, according to the formula for a hemiellipsoid as follows (9):

$$V = \frac{1}{2}(4\pi/3)(l/2)(w/2)(h) = 0.5236 lwh$$

Any deaths occurring during the first 21 days of observation were considered drug related. The posttreatment observation period extended for 33 days, after which the animals were sacrificed.

**In Vivo Testing Using Nonpalpable WD PaCa Tumors.** Twenty hamsters were implanted with tumor fragments of uniform size, approximately 2 cu mm. One week after implantation, at a time when the tumors were not palpable, the animals were divided into a control group and an ADR treatment group of 10 each. The tumors were measured and volumes were determined (as described above) 4 times/week, once the tumor became palpable.

In a second experiment, 44 hamsters were similarly implanted, with treatment begun on Day 7, and were measured as above. The treatment groups of 11 each were untreated controls, MMC, SZN, and FAM.

**In Vivo Testing Using PD PaCa.** LSH hamsters were inoculated with  $3 \times 10^6$  PD PaCa cells/animal, 10 animals/group (Day 0). Twenty-four hr later (Day 1), drug therapy was initiated with ADR, MMC, SZN, or no treatment (controls). Animals were checked twice daily to determine the time of death.

**In Vitro Drug Sensitivity Assays Using WD PaCa and PD PaCa Cells.** The *in vitro* assays were carried out after preliminary experiments

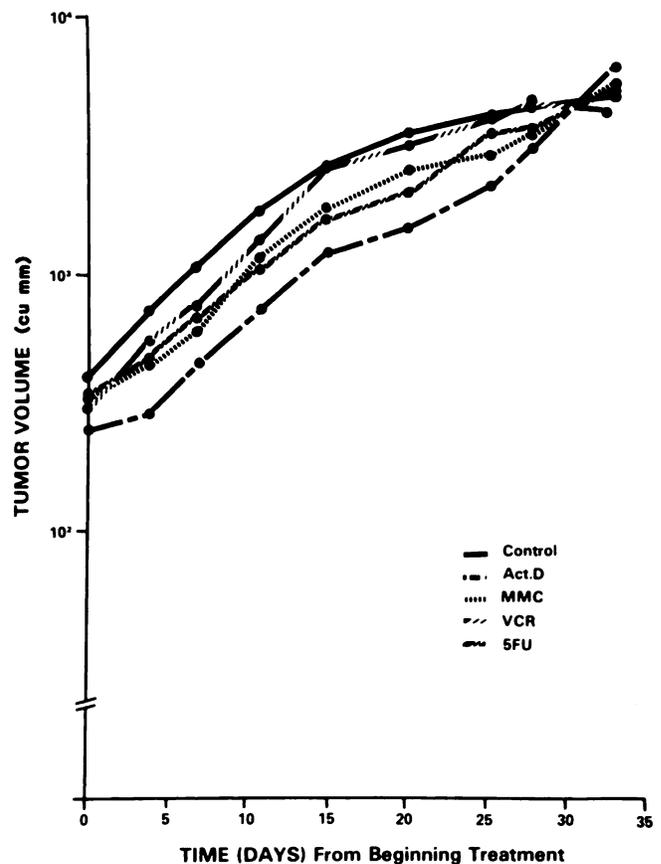


Chart 2. WD PaCa tumor growth curves showing no significant differences between the control group (same controls as shown in Chart 1) and groups treated with actinomycin D (Act. D; 0.24 mg/kg on Day 1), MMC (1.2 mg/kg on Day 1); vincristine (VCR; 0.6 mg/kg on Day 1) and 5-fluorouracil (5FU; 30 mg/kg on Days 1 to 7).



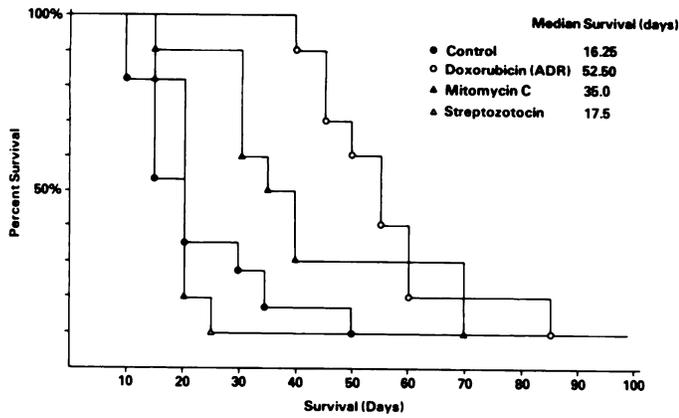


Chart 5. Survival curves of hamsters inoculated i.p. with PD PaCa,  $3 \times 10^6$  cells/animals on Day 0, and treated i.p. with ADR (3.75 mg/kg on Day 1), MMC (2.5 mg/kg on Day 1), SZN (50 mg/kg on Day 1), or no therapy (controls).

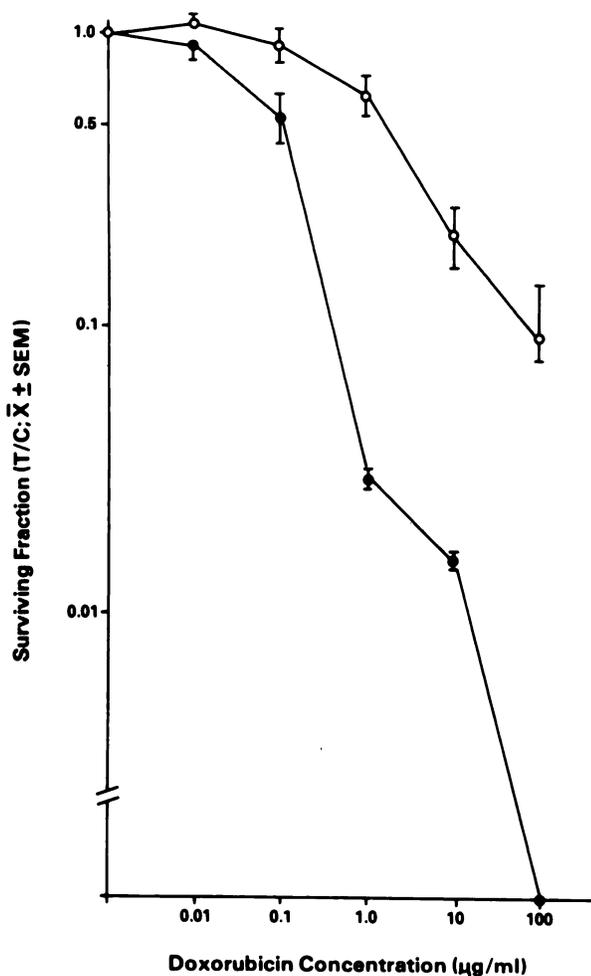


Chart 6. *In vitro* dose-survival curves showing a marked difference in sensitivity of WD PaCa (O) and PD PaCa (●) cells to ADR.

The failure to observe antitumor activity of drugs against palpable WD PaCa may have been due to the large tumor burden. We therefore decided to screen ADR, MMC, SZN, and FAM against nonpalpable tumors. The results of these experiments are depicted in Charts 3 and 4. Again, regression, significant slowing, and a  $T/C \leq 0.42$  were not observed when nonpalp-

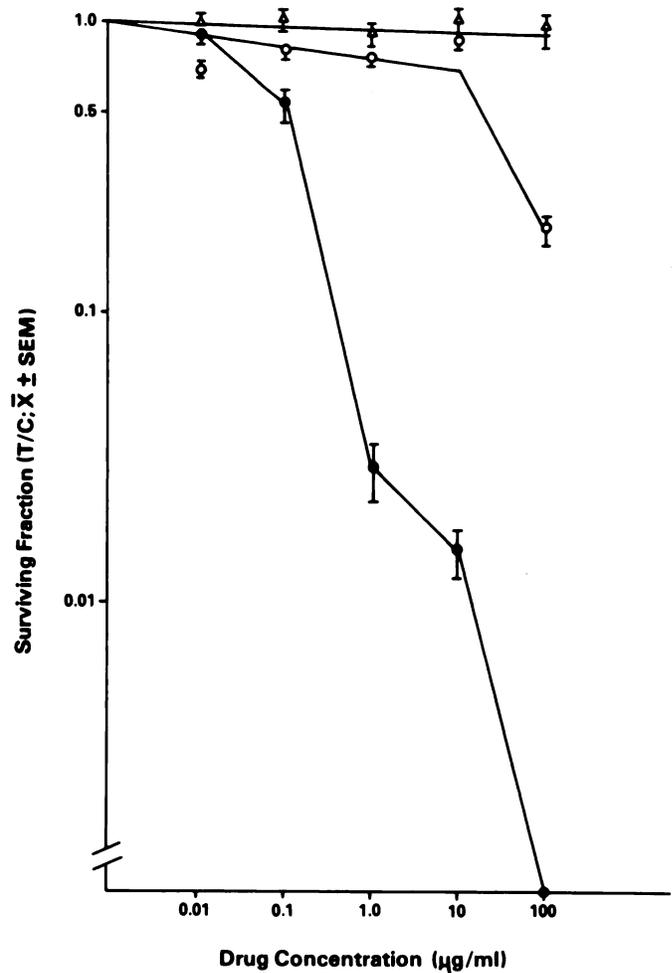


Chart 7. *In vitro* dose-survival curves of PD PaCa cells in response to ADR (●), MMC (O), and SZN (Δ).

able 1-week WD PaCa tumors were treated with ADR, MMC, SZN, or FAM.

***In Vivo* Testing Using PD PaCa.** PD PaCa displayed definite chemosensitivity to ADR (see Chart 5). Median survivals in days were: controls,  $16.25 \pm 4.15$  (S.E.); ADR,  $52.5 \pm 3.95$ ; MMC,  $35 \pm 7.9$ ; and SZN,  $17.5 \pm 1.3$ . ADR produced a significant prolongation of survival in comparison to controls ( $p = 0.003$ ), whereas MMC and SZN did not ( $p = 0.097$  and  $0.57$ , respectively).<sup>5</sup>

***In Vitro* Drug-Sensitivity Assays Using WD PaCa and PD PaCa.** Drug sensitivity was assessed *in vitro* to determine intrinsic differences in the 2 cell lines, independent of the problems of drug delivery. The ADR dose-survival curves summarized in Chart 6 show a marked difference in the sensitivity of the tumor models to ADR. Significant differences are found (via *t* test comparisons) at all dose levels higher than  $0.01 \mu\text{g/ml}$ . Chart 7 shows the dose-survival curves for ADR, MMC, and SZN against PD PaCa, which follow the same pattern of response as seen *in vivo*.

<sup>5</sup> The authors recognize that a *t*-test comparing the median survival of the MMC group to controls would yield a significant difference ( $p < 0.001$ ), and therefore, modest sensitivity to MMC may be present. However, the computer-assisted method used to assess significance takes the entire survival curve into account and provides a more conservative estimate of significance.

## DISCUSSION

In the present study, we have begun to characterize the chemosensitivity of 2 recently available hamster tumor models, WD PaCa and PD PaCa. ADR (at the LD<sub>10</sub> and LD<sub>50</sub> levels), 5-fluorouracil, MMC, actinomycin D, cyclophosphamide, methotrexate, and vincristine were ineffective against palpable WD PaCa tumors. Nonpalpable WD PaCa tumors were resistant to ADR, MMC, SZN, and FAM. In contrast, PD PaCa shows marked sensitivity to ADR, both *in vivo* and *in vitro*, with relative resistance to SZN and perhaps borderline sensitivity to MMC.

Ours is the first report to provide a chemotherapy response profile for the 2 recently described animal models of pancreatic adenocarcinoma. Since human cancer is known to be heterogeneous, there are distinct benefits to be derived from the use and comparison of a variety of tumor models. Our data would indicate that the WD PaCa represents a model exhibiting relative refractoriness to chemotherapy, which is similar to the common human situation (where objective responses to single agents are in the range of 20 to 28% for the most effective agents) (13). WD PaCa could be exploited preclinically to test other agents, combinations of agents, or newer innovative therapy. Comparison of WD PaCa and PD PaCa may shed light on the mechanisms of chemotherapy sensitivity *versus* resistance. Furthermore, future preliminary screening can more efficiently be conducted *in vitro* for both tumor models.

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